MOUTHRINSES

physico-chemical properties and short term clinical efficacy

J.F. Perdok

STELLINGEN BEHOREND BIJ HET PROEFSCHRIFT

MOUTHRINSES

Physico-chemical properties and short term clinical efficacy

door

Johan Perdok

1. Met behulp van *in vivo* gemeten randhoeken op tandglazuur kan geen uitspraak worden gedaan omtrent de plaque accumulatie.
Dit proefschrift

2. Vanuit thermodynamisch oogpunt bezien kan door verlaging van de hoeveelheid natriumlaurylsulfaat (SLS) in mondspoelmiddelen de effectiviteit hiervan worden vergroot.
Dit proefschrift

3. Bij de bestudering van de *in vivo* effecten van mondspoelmiddelen geven de *in vitro* resultaten slechts zeer beperkte informatie.
Dit proefschrift

4. Het deelnemen aan tandheelkundig modelonderzoek bevordert de mondhygiëne van de deelnemers.
Dit proefschrift

5. Het verdient aanbeveling de concentratie van chloorhexidine in mondspoelmiddelen in Europa terug te brengen tot 0.12%.
Segreto et al., J Period Res 1986; suppl: 33-36

6. Mondspoelmiddelen zijn niet in staat plaque te beïnvloeden in moeilijk toegankelijke interdentale ruimten.

7. De opmerking in het artikel "Het fluoridefront brokkelt af" dat onderzoeksresultaten laten zien dat lager fluoride gebruik is verantwoord, is in het geheel niet onderbouwd.
Chemisch Magazine, p. 4 januari 1991

8. Het gebruik van het woord "fluor" in de literatuur en in de pers wanneer fluoride wordt bedoeld maakt de auteur bijna automatisch tot een tegenstander van het gebruik van fluoride.

9. Door de toename van het aantal "creditcards", zal de uitdrukking "geld moet rollen" aan betekenis inboeten.

10. Het veelvuldig gebruik van de termen "zo optimaal mogelijk" en "zo maximaal c.q. zo minimaal mogelijk" laat zien dat de Nederlander zijn taal niet optimaal gebruikt.

11. Gezien de heftige dermatologische reacties die azokleurstoffen als sunset yellow en tartrazine soms veroorzaken, zou de toevoeging van deze stoffen aan voedings- en geneesmiddelen moeten worden ontraden.

12. Bij het gebruik van het woord "light" in de voedingsmiddelen reklame, zou bij veel slanke lijners een "lichtje" moeten gaan branden.

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Physico-chemical properties and short term clinical efficacy

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Johan

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CHAPTER 1

INTRODUCTION

1.1. INTRODUCTION

Dental caries and chronic inflammatory periodontal disease are the two most widespread diseases in mankind.[1] Both diseases originate from the presence of the dental plaque, which forms on smooth tooth surfaces and in fissures and pits. Plaque is variable in composition, but generally consists of salivary components, desquamated epithelial cells and bacteria. Several microorganisms have been found capable of inducing caries and gingivitis, from which *Streptococcus mutans, Lactobacilli* spp. and *Actinomyces* spp. are considered to be the most cariogenic.[2] *Bacteroides gingivalis* and spirochetes have been found in relation with periodontal disease.[3]

Plaque formation starts with the initial adhesion of single microorganisms, predominantly *Streptococcus sanguis*, *Streptococcus mitis* and *Veillonella parvula*, to the tooth surface.[4] The initial adhesion phase is to a large extent governed by the enamel surface free energy and surface roughness;[5-7] therefore measures to discourage plaque formation based on lowering the enamel surface free energy have been proposed.[8,9]

Most common methods for mechanical plaque removal are the use of toothbrushes and toothpastes, toothpicks and dental floss. Unfortunately, only a few people can obtain a plaque free dentition using the methods mentioned. Therefore the addition of therapeutic compounds to toothpastes and to rinses is important. Therapeutic compounds added to toothpastes are e.g. fluorides to prevent demineralisation and to stimulate remineralisation, pyrophosphate for tartar prevention and anti-microbials like e.g. chlorhexidine, triclosan, to realize plaque reduction.

Although toothpastes are still most widely used to prevent caries and periodontal diseases, mouthrinses gain more and more popularity as an addendum to common daily oral hygiene, particularly since they provide a fast and convenient method to create a fresh breath. Mouthrinses can also be used as a vehicle to administer anti-bacterial compounds such as chlorhexidine and sanguinarine.[10] Furthermore, mouthrinses can be helpful after oral- and periodontal surgery when toothbrushing is impossible, and can be very effective to obtain a good oral hygiene in special risk groups like disabled and mentally retarded people. Mouthrinses can be employed either after or before toothbrushing. Possible working mechanisms of mouthrinses are summarized in Fig. 1.1.

The number of studies, that have been carried out to evaluate the overall clinical efficacy of mouthrinses, is very large. In most mouthrinse investigations one or two rinses are compared with a control rinse or water. In such studies a positive effect of the test rinse is in general obtained.

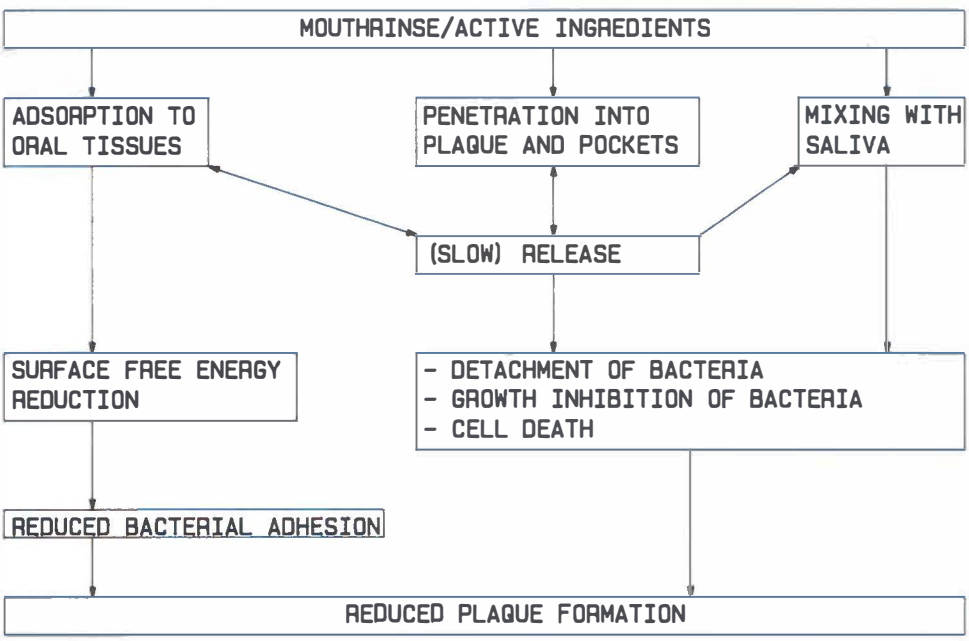

Fig 1.1. Possible working mechanisms of mouthrinses.

The therapeutic effect of a mouthrinse is dependent on numerous factors: the effects of its active ingredients *in vivo*, the permeability and solubility of the agents, and the substantivity (the prolonged association of drug and substrate, greater than would be expected on basis of simple mechanical deposition).[11] The administration mode, salivary and crevicular flow will also influence the active ingredient efficacy.[11] A good penetration of the mouthrinse into the plaque or gingival pockets, as well as into pits and fissures is required in order to bring the action of a rinse where it is needed. Furthermore, in order to give a long lasting rinse effect, the Minimal Inhibitory Concentration (MIC) should be maintained in the oral cavity as long as possible.[11] Adsorption of active components to and subsequent slow release from oral tissues, must ensure such an effect.

Recently, industrial research and development is also directed towards products that decrease the tooth surface free energy[12], therewith reducing the thermodynamic driving force for bacterial adhesion.[9]

1.2. AIM

The aim of this study is to investigate physico-chemical properties responsible for the clinical effectiveness of commercially available mouthrinses, as well as their overall - short term - clinical efficacy. The physico-chemical parameters of the rinses studied include: adsorption to enamel, penetration, and tooth surface free energy alteration.

In vivo the relative efficacy of the rinses is established by measuring the Plaque Index (PI), the Gingiva Index (GI), the Planimetric Plaque index (PP), the tooth surface free energy and the microbial composition of the plaque.

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CHAPTER 2

LITERATURE SURVEY

2.1. INTRODUCTION

Several studies have demonstrated the relationship between the presence of plaque on teeth and the development of caries and periodontal diseases.[1] Oral hygiene programs are focussed on plaque removal. Only few people however, are capable to create a plaque free dentition. Most people are satisfied already with a fresh breath and a good taste, and consider these effects the most important after toothbrushing. With the aid of effective anti-plaque compounds, a good oral health can be obtained, especially when the active ingredients are incorporated in a mouthrinse or in a toothpaste. Rinsing is an attractive and simple preventive method. It should be noted however, that mouthrinsing alone without brushing is not effective enough to obtain a plaque free dentition; especially interdental and subgingival plaque is not removed by any rinse. A mouthrinse is attractive as anti-plaque treatment before or after toothbrushing as well as in special situations like intermaxillary fixation or after periodontal surgery.

This chapter reviews a number of anti-plaque compounds which can be or are used in mouthrinses. They are divided in antibiotics, guanides, surface active compounds, enzymes, ammonium compounds and inorganic fluorides.

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2.2. HISTORICAL REVIEW[2]

For thousands of years mankind has been suffering from caries, periodontitis and bad breath. On Egyptian papyri from 3800 years B.C., translated by Eber in 1873, 11 recipies were found as remedies against toothache, fistula, swellings and gum diseases. They include pastes and powders as well as mouthrinses. The ingredients were extracted from plants or plant roots. The ancient Greek are known using mouthrinses to combat toothaches; the ancient Romans especially appreciated beautiful and clean teeth. Mouthrinses were never really brought in vogue in history. In early days various ingredients were added to wine in order to rinse the mouth. In 1728, Pierre Fauchard wrote his knowledge on the subject in 'Le chirurgien dentiste ou traité des dents' recommending against toothache: 'rinse the mouth in the morning and in the evening with a few spoons of freshly produced urine of a healthy person' and ensured his patients quick succes. Afterwards Pasteur in 1857 described the role of microorganisms in infectious diseases. The English surgeon Lister prevented infections during operations by using a 50% carbolic solution.

W.D. Miller, in his 'Human organisms of the mouth' recommended the use of mouthrinses against plaque-accumulation, and added sublimate ($HgCl_2$) as active ingredient. At the same time he warned against injudicious use.

Around 1960 the interest in mouthrinses strongly increased. Löe and Rindom Schiøtt were the first authors who described on a scientific basis the effects of chlorhexidine in a mouthrinse, after which a seemingly endless flow of papers on this subject has been published.[3]

2.3. MOUTHRINSES

A mouthrinse is an outstanding vehicle to administer drugs. Various rinses can differ considerably in composition but there are a few basic ingredients which most

products have in common. These are water, alcohol, taste compounds and colouring agents.[4] Other ingredients can be detergents and specific therapeutic compounds. In general alcohol is added to dissolve water insoluble components and for taste requirements. Detergents are added for two important reasons:

- lowering the liquid surface tension. The surface tension is lowered so that droplets of the rinse can easier spread over the contact surface; the contact area between rinse and tooth is therefore increased.
- increase of the cleaning capacity. Particles in solution are surrounded by the detergent, and form micells. As a result particles are removed more readily. In this way plaque can be partially removed from the tooth surface. [5,6]

2.3.1. Distribution of a mouthrinse in the oral cavity

Weatherell *et al.*[7,8] found that the oral cavity is highly compartimentalised, resulting in an unequal distribution of compounds through the oral cavity as a whole. When a fluoride tablet is placed in the lower buccal sulcus at one side of the mouth, there is little or no flow of fluoride to the other side. In the upper jaw there is a migration effect to the contra-lateral side, attributed to the salivary flow from the parotid gland. The clearance of fluoride from the sublingual area and the postcanine area is fast. Differences in clearance from the various compartiments are mainly caused by the differences in salivary flow from the parotid gland and the sublingual and submaxillary glands. Retention of fluoride occurs in the buccal central part of the maxilla because the salivary flow is lowest in that part of the mouth.

Although these experiments only have been carried out with fluoride, we assume that a similar behaviour occurs for any dissolved compound in mouthrinses in the oral cavity.

2.4. EFFECTIVENESS

2.4.1. Substantivity

There are several factors which play an important role in the effectiveness of active components in mouthrinses.[9] Compounds used in a mouthrinse need to have a low acute and chronic toxicity, a convenient potency and, for topical use, a low solubility in saliva. The intrinsic efficacy of a drug is the fraction of a maximum achievable effect that can be obtained. Maximum intrinsic efficacy of an anti-bacterial agent would be its ability to inhibit bacterial growth completely. The intrinsic efficacy of thymol for instance is low, whereas the intrinsic efficacy of chlorhexidine is very high. The most important factor of an active component is its "substantivity", meaning a prolonged association between material and substratum, greater than would occur by simple mechanical retention. Most therapeutics demonstrate non-specific adsorption to tissues by van der Waals-forces, hydrophilic attraction or covalent bonding. Adsorption can be responsible for the formation of a reservoir, from which the drugs are slowly released. There are great differences in substantivity of various drugs. From a chlorhexidine rinse 30% of the total amount adheres to oral tissues, while less than 1% of a sodiumfluoride solution is bound.[10,11] A high substantivity does not necessarily indicate a good clinical result, because substantivity can also be too high, resulting in a permanent adsorption, preventing slow release action of the drug. The anti-microbial activity of cetylpyridiniumchloride e.g. is better than that of chlorhexidine, whereas its plaque inhibiting effect is much smaller. This difference is probably caused by differences in retention time of the active components in the oral cavity as related to their adsorption/desorption behaviour to oral tissues.[12]

2.4.2. Comparison of *in vivo* and *in vitro* effects

When studying anti-plaque effects of a mouthrinse, it is important to realise that anti-bacterial effects are not identical to anti-plaque effects. Gjermo *et al.* found no correlation between anti-bacterial effects *in vitro* and anti-plaque effects *in vivo*.[13]

2.5. CHLORHEXIDINE AND DERIVATIVES

Several guanides being derivatives of urea possess anti-bacterial properties. Chlorhexidine is the most famous representative of this group, other compounds being sanguinarine, alexidine and hexetidine.

2.5.1. Chlorhexidine

Chlorhexidine (1,6-bis-p-chlorophenylbiguanidohexane) is a bis-biguanide introduced 40 years ago.[14] Bis-biguanides possess most potent anti-bacterial properties when the structure of the molecule contains a hexamethylene bridge with terminal aryl groups e.g. the 4-chlorophenyl groups in chlorhexidine. The anti-bacterial properties are also dependent on the geometry of the molecule. The affinity of bis-biguanides for enamel is pH dependent, which is an indication for the presence of electrostatic interactions between these compounds and enamel.[14]

The working spectrum of chlorhexidine is wide: it is active against Gram-negative and Gram-positive microorganisms as well as yeasts. The toxicity of chlorhexidine is low which is an important contribution to its widespread use in medicin. Its first application in dentistry took place in 1959 in endodontics.[15] In 1970 chlorhexidine was employed for the first time as an anti-plaque agent in humans.[3] Since that time, many publications appeared on the effect of chlorhexidine on plaque and gingivitis.[16-22]

Chlorhexidine is a cationic compound. Dissolved in water it becomes positively charged, due to the four =N-H groups in the molecule which attrack H^+ ions. This positive charge causes an interaction with negatively charged groups on bacterial cell walls and with pellicle coated oral surfaces. The interaction with the bacterial cell wall causes an increased cell permeability; chlorhexidine can penetrate into the cytoplasm resulting in cell death.[23]

Chlorhexidine is an active anti-microbial agent in the oral cavity during prolonged time intervals. Due to the differences in charge mentioned above, a "depot" is formed from which it is slowly released.[24] The retention of chlorhexidine to the oral tissues has been measured with the C-14 method.[25] After a 10 ml Hibitane® (containing 0.2% chlorhexidine) rinse, 34% retention was found after 3 minutes. A quick decrease in the chlorhexidine concentration has been observed in the first hours following the rinse; 24 hours later it still can be detected in saliva.

2.5.2. Application and dosage

The most common dosage of chlorhexidine in mouthrinses in Europe is a 0.2% solution,[26-30] a safe and effective concentration. In the USA the chlorhexidine concentration allowed by the Food and Drug Administration (FDA) is a 0.12% solution. In a few studies it has been demonstrated that there are no significant differences between a 0.2% and a 0.12% solution on the prevention of plaque and gingivitis.[31,32] A 0.12% solution seems even more ideal against plaque and gingivitis. Patients will probably be more cooperative if the low dosage is used, because side effects will be smaller.

In Europe a 0.2% chlorhexidine containing mouthrinse is often employed, for e.g. early stages of Acute Necrotizing Ulcerative Gingivitis (ANUG) or acute gingivitis. Application in a spray or a rinse is indicated when toothbrush application is not possible (e.g. intermaxillar fixation). A local application with gels as well has been studied extensively.[33,34]

2.5.3. Effectiveness and side effects

Chlorhexidine has a broad working spectrum as mentioned previously in 2.5.1., and is capable to prevent plaque growth completely at application sites for a given period. The effect of chlorhexidine on bacteria is strongly dependent on the type of bacteria considered. *Streptococcus mutans* is more sensitive for chlorhexidine than e.g. *S. sanguis* or *Actinomyces* spp.[35] If a chlorhexidine therapy is abandonned, it appears that the amounts of *S. mutans* is still significantly lower in fissures after 21 days. However, the amount of *S. sanguis* and *A. viscosus* reached the original level already after 7 days.[36]

There are some major disadvantages of chlorhexidine applications: dark staining of teeth, composite restaurations and tongue have been frequently reported as a consequence of a long term chlorhexidine application in some patients. Desquamation of epithelial cells and soreness of the oral mucosa (lesions on tongue, gingiva and the lips) have been observed. Therefore this effective anti-microbial cannot be prescribed for long term use.[36]

2.5.4. Compounds analogous to chlorhexidine

Sanguinarine, an ingredient of Veadent®, is another bis-biguanide. Sanguinarine is a plant alkaloid, and is extracted from the Sanguinaria Canadenses. It has anti-bacterial properties,[37] and inhibits plaque and gingivitis development when compared to a placebo.[38] However, the use of chlorhexidine gives a significantly higher inhibition of plaque growth than Veadent®.[39,40] Oraldene® is a mouthrinse containing 0.1% hexetidine. Both the Plaque Index (PI) as well as the Gingiva Index (GI) were significantly higher after the use of a hexetidine containing rinse, as compared to a chlorhexidine rinse so it is less effective.[41] The negative effects of discoloration of restaurations and irritation of the tongue previously found for chlorhexidine have also been observed after hexetidine applications.

2.6. SURFACTANTS

2.6.1. Introduction

A surfactant is a compound that alters the surfactive properties of a solution.[42] Most oral hygiene products contain surfactants either to improve the dissolution properties, as a preservative or to create foam. Surfactive properties play an important role in the deposition and adherence of bacteria to the tooth surfaces. A surfactant adsorbed from e.g. a rinse can be used to decrease the surface free energy of a solid such as enamel. From low energetic surfaces bacteria can be removed more easily. Surfactants can also inactivate other compounds in a rinse, e.g. those compounds added for taste requirements.

2.6.2. Surfactive compounds

In dentistry three groups of surfactive compounds are important: anionic, cationic and nonionic compounds.[42]

Anionic surfactants are incorporated in dentifrices and mouthrinses for their ability to create a foam. Sodiumlaurylsulphate is a typical anionic surfactant often used in oral hygiene products. It has no direct taste, a high foam creating property, low toxicity and it is easy to process. This surfactant is responsible for a sometimes unpleasant taste after toothbrushing if e.g. an orange is consumed. Sodiumlauryl-sarcosinate is recommended in mouthrinses containing cationic anti-bacterial compounds.

Cationic surfactants are used only as anti-bacterial compounds; chlorhexidine and cetylpyridiniumchloride belong to this group. Nonionic surfactants are used for taste effects; they do not possess anti-bacterial properties.

2.7. OTHER COMPOUNDS USED IN MOUTHRINSES

2.7.1 Antibiotics

Several studies have been carried out to investigate the effect of antibiotics on plaque- and gingivitis reduction.[43-46] Vancomycin, polymyxine B and tetracycline are capable to reduce the amount of plaque.[47,48] Vancomycin, however, induces a Gram-negative flora, which can result in the development of gingivitis. Tetracyclins have a broad working spectrum and therefore e.g. *Candida albicans* infection can develop in due time. Discoloration of developing teeth can occur when tetracyclines are used during pregnancy or lactation. Erythromycin reduces the amount of plaque with 35%.[49] Side effects as diarrhoea, vomiting and the development of resistant micro-organisms have been reported after erythromycin treatments. Metronidazole is a specific antibiotic against anaerobic microorganisms,[50] and is commonly used against *Trichomonas Vaginalis* infections. Oral Gram-negative bacteria induced periodontitis can be succesfully treated with metronidazole.[51-55] It is a safe antibiotic with a low effective dose and a low resistance development.

Summarizing, antibiotics are not suitable for long term use in mouthrinses against plaque-accumulation; the main disadvantages being:

- microorganisms can develop a strong resistance against the compound, making it impossible to employ the agent in case of serious infections;
- superinfections can develop e.g. *C. albicans*;
- patients can easily develop hypersensivity;
- the plaque changes might result in periodontitis.

Antibiotics in a rinse, however, can be very useful for short-term therapy before and after periodontal surgery.

2.7.2. Enzymes

Dental plaque consists for 10 - 20% of polysaccharides, produced by micro-organisms. These polysaccharides accumulate outside the bacterial cell wall and play an important role in the adhesion of the cells to each other and to the tooth surfaces. These extra cellular polysaccharides mainly consist of dextranes. One can expect therefore that plaque-accumulation can be influenced by destruction of these dextranes with dextranase. A dextranase containing mouthrinse has, however, not yet been found capable to influence plaque-accumulation or gingivitis development compared to a placebo.[54,55]

The enzyme lactoperoxidase catalyses the oxydation of thiocyanate ($OCNS^-$) from saliva together with H_2O_2 produced by bacteria. The reaction product is $OCNS^-$, and this ion might inhibit bacterial growth.[56] In a 50 days clinical study, the use of an enzyme containing toothpaste has been reported to cause a significantly lower plaque index and an improved condition of the gingiva.[57,58] Adding enzymes to a toothpaste or mouthrinse can in principle be useful to inhibit plaque growth.

There are several reasons why enzymes cannot be used routinely: enzymes are proteinaceous compounds and are de-activated in the oral cavity. The enzyme action is in general very slow and consequently needs a long retention period in the mouth. Another disadvantage is specificity: enzymes are active only against one given group of molecules.

2.7.3. Quaternary ammonium compounds

Quaternary ammonium compounds possess anti-bacterial properties and can be used as bacteriostatic or bactericidal agents. They form positively charged ions which can bind to the negatively charged mucosa, microorganisms or pellicle coated teeth. Quaternary ammonium compounds are surfactive agents and have a quite long oral clearance period. Aminefluorides and cetylpyridiniumchloride belong to this group.

2.7.3.1. Aminefluorides

Aminefluorides are quite often used in dentistry to prevent caries. The surfactive molecule contains a polar group and a long hydrocarbon chain. The positively charged group of the aminefluoride can bind easily to the (negatively charged) tooth surface, yielding a high fluoride concentration on the tooth surface; the fluoride can subsequently be incorporated in the hard tissues.[59] Aminefluorides, like other fluorides, at a given dosage have anti-bacterial properties.[60-62] Sodiumfluoride is bacteriostatic only at very high concentrations, while aminefluorides are already bactericidal in lower concentrations. It has been demonstrated that aminefluorides lower the enamel surface free energy.[63] A low surface free energy results in a weaker binding of bacteria to the tooth surface and easier plaque removal.[64]

2.7.3.2. Cetylpyridiniumchloride

This is a quaternary ammonium compound which easily binds to surfaces in the oral cavity. With respect to a placebo, a 0.1% cetylpyridiniumchloride solution causes a significant plaque reduction after 10 days.[65]

The effect of cetylpyridiniumchloride is lower than expected on theoretical grounds because it is inactivated by food components, calcium ions (dairy products) and by serum proteins. Prolonged use can cause discoloration of oral tissues. Various authors found no plaque reduction with a 0.025% cetylpyridiniumchloride solution.[66] However a combination of 0.025% cetylpyridiniumchloride and 0.005% domiphen-bromide seems to cause a plaque reduction of 38%.

2.7.3.3. Inorganic fluorides

Fluorides have been employed in preventive dentistry extensively because they influence caries prevalence directly. A 0.02 mM (equivalent to 0.38 ppm F^-) fluoride

containing solution causes a change in the metabolism of oral bacteria in such a way that the acid production is decreased. Fluorides can inactivate enolase, an enzyme in the glucose breakdown pathway. As a result the conversion from glucose to lactic acid is inhibited; moreover the transport of glucose through the cell is inhibited by fluoride. Stannousfluoride possesses more complex anti-microbial properties.[67-70] When applied in very low concentrations (about 2.5 ppm) for a long time or in high concentrations for a short time (about 25000 ppm) the surface free energy of enamel is reduced.[71] A combination of aminefluoride and stannousfluoride is known to cause a significant plaque reduction with respect to a placebo rinse.[72]

2.8. ADDITIONAL REMARKS

From the literature on mouthrinses it is apparant that there is a lack of information on:

- the relative efficacy of rinses *in vivo*,
- how active ingredients in a rinse interact with plaque and with the hard tissues,
- discoloration effects of the active ingredients in rinses.

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CHAPTER 3

SURFACE FREE ENERGIES AND ELEMENTAL SURFACE COMPOSITIONS OF HUMAN ENAMEL AFTER APPLICATION OF COMMERCIALLY AVAILABLE MOUTHRINSES

3.1. ABSTRACT

In this study the adsorption of therapeutically active components from six commercially available mouthrinses on ground and polished enamel with and without adsorbed salivary constituents, is monitored by contact angle measurements and X-ray Photoelectron Spectroscopy (XPS). Human enamel samples were treated with mouthrinses containing chlorhexidine (Peridex®), stannousfluoride/aminefluoride (Meridol®), thymol/benzoic acid (Listerine®), sanguinarine (Veadent®), sodium-fluoride (Prodent®) or cetylpyridiniumchloride (Merocet®). XPS results indicated a sizeable adsorption of both active and non-active components for all products. After the various treatments all enamel surface free energies increased, except for the stannousfluoride/aminefluoride containing mouthrinse. It is suggested that non-active components in the products, cause this increase in surface free energy. Despite this thermodynamically unfavourable increase in surface free energy, all rinses have a more or less pronounced plaque reducing effect, indicating that the unfavourable surface characteristics are overruled by the anti-bacterial properties of the components. Replacement of non-active components by less adsorbing surfactants, could therefore increase the efficiency of the products tested.

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3.2. INTRODUCTION

Dental caries and chronic inflammatory gingivitis are the most widespread diseases in man, originating from the adhesion of dental plaque to tooth surfaces.[1] Mouthrinses can be useful in preventing the formation and reduction of dental plaque, although they will probably never replace toothbrushing completely. However, mouthrinses gain popularity as an addendum to daily toothbrushing. Furthermore, rinses are useful for patients which are unable to brush. Normal basic ingredients of commercially available mouthrinses are, besides their therapeutically active components, ethanol, humectants, flavours, surfactants and water. Surfactants are used for their ability to make a foam, which is emotionally desirable during the use of the product and assist removal of oral debris by detersive action.[2] Currently a wide variety of therapeutically active components are added to the basic ingredients of a mouthrinse in order to create bactericidal effects or to stimulate enamel remineralisation. Mouthrinses containing chlorhexidine are considered to be the most effective in reducing plaque formation[3,4] as compared to mouthrinses containing e.g. sanguinarine[5] or cetylpyridiniumchloride.[6] Mouthrinses based on aminefluorides and/or stannousfluoride (or sodiumfluoride) are thought to be effective in enhancing enamel remineralisation and to reduce enamel demineralisation.[7,8]

In this paper we focus on the ability of mouthrinse components to adsorb on the enamel surface. X-ray Photoelectron Spectroscopy (XPS) is an elegant technique to study the composition of the outer surface of teeth,[9] but has been used surprisingly little in dentistry.[10] With XPS the elemental composition of the outer tooth surface can be monitored over a depth up to 3-4 nm. Recently the ability of chlorhexidine and cetylpyridiniumchloride[11,12] to adsorb on the enamel surface has been demonstrated by a combination of XPS and contact angle measurements. Contact angle measurements and subsequent calculation of surface free energies present an extremely sensitive method to detect changes at a surface, because the depth of information is confined to the outer 0.2 to 0.4 nm. Compared to XPS however,

contact angles have a disadvantage since they do not yield information on the chemical composition of the surface.

It is the aim of this paper to compare the *in vitro* adsorption of active components of six commercially available mouthrinses to ground and polished human enamel with and without adsorbed salivary components by means of both contact angle measurements and X-ray Photoelectron Spectroscopy.

3.3. MATERIALS AND METHODS

Small pieces of enamel (3x4 mm) cut from the buccal surfaces of freshly extracted human incisors were ground on siliconcarbide paper (1200 grit) under running tap water, polished with a slurry of Al_2O_3 powder (particle diameter 0.05 μm) in distilled water and subsequently ultrasonically cleaned to remove adhering polishing particles. This procedure resulted in the removal of approximately 50 - 100 μm from the anatomical tooth surface. The stylus surface roughness of thus obtained surfaces is smaller than 0.1 μm, required for surface free energy determination from measured contact angles.[13]

Whole saliva from a pool of ten people was stimulated by having them chew on Parafilm® (American Can Company, Greenwich CT, U.S.A.). The saliva was collected on ice and immediately cleared by centrifugation for 10 minutes at 5000 *g*. The clear supernatant was quickly frozen in liquid nitrogen and stored at -20°C. Before use aliquots were thawed at room temperature.

Table 3.1 lists all mouthrinses employed for this study together with their therapeutically active components, primary objectives and manufacturers. All products were purchased commercially.

Table 3.1. Commercially available mouthrinses used in this study, their therapeutically active components and objectives as indicated by the manufacturer.

Tradename (abbreviation)	Therapeutically active components	Objective	Manufacturer
Peridex® (Pe)	Chlorhexidine 0.12% $C_{22}H_{38}N_{10}Cl_2$	anti-plaque	Procter & Gamble Cincinnati, U.S.A.
Meridol® (Md)	Aminefluoride 297 (125 ppm) $C_{27}H_{66}N_2\ O_3F_2$ Stannousfluoride (125 ppm) SnF_2	anti-plaque anti-caries	GABA, Basel, Switzerland
Listerine® (L)	Benzoic acid $C_7H_6O_2$ Thymol $C_{10}H_4O$ Methyl Salicylate $C_8H_8O_3$	anti-plaque	Lambert Chemical Co., Eastleigh, U.K.
Veadent® (V)	Sanguinarine 0.03% $C_{20}H_{14}NO_5$	anti-plaque	Vipont Lab. Inc., Ft. Collins, U.S.A.
Prodent® (Pr)	Sodium fluoride 0.05% NaF	anti-caries	Intradal Amersfoort The Netherlands
Merocet® (Me)	Cetylpyridinium chloride 0.05% $C_{21}H_{38}NCl$	anti-bacterial	Merell Pharma-ceuticals Ltd., U.K.

Salivary constituents were adsorbed on the enamel (with and without adsorbed mouthrinse components present), by putting each enamel sample in 5 ml saliva for 90 minutes, after which the samples were dipped once in distilled water to remove loosely bound components.

The mouthrinses were applied to the enamel (with and without adsorbed salivary constituents present) by putting the samples in 5 ml mouthrinse for 2 minutes. Subsequently the samples were dipped once in distilled water. All measurements were done on 5 different enamel blocks.

In summary, 200 enamel blocks were used from which 10 were only ground and polished, while 10 were used with a saliva coating only (+S); 60 pieces were treated with a mouthrinse only (denoted as +Pe, +Md, +L, +V, +Pr and +Me for respectively Peridex®-, Meridol®-, Listerine®-, Veadent®-, Prodent®- and Merocet® application), 60 pieces received a saliva coating followed by application of a mouthrinse (denoted as +S+Pe, +S+Md, +S+L, +S+V, +S+Pr and +S+Me), and 60 pieces were treated with a mouthrinse followed by adsorption of salivary constituents (denoted as +Pe+S, +Md+S, +L+S, +V+S, +Pr+S and +Me+S). One half of all enamel pieces was used for the contact angle measurements, the other half for the XPS analysis.

Advancing type contact angles (Θ) with liquids of known dispersion (γ_l^d) and polar (γ_l^p) surface free energies (water, six water/n-propanol mixtures and α-bromonaphthalene) were measured at 25°C employing the sessile drop technique. Prior to the contact angle measurements the samples were dried for 90 minutes at 25° C, to remove loosely bound water. During the measurements care was taken not to wet the same spot twice. The dispersion (γ_s^d) and polar (γ_s^p), surface free energies of the enamel surfaces were estimated by least square fitting a series of such contact angle data to the geometric mean equation[14]

$$\cos\theta = -1 + 2(\gamma_s^d \cdot \gamma_l^d)^{1/2} \cdot \gamma_l^{-1} + 2(\gamma_s^p \cdot \gamma_l^p)^{1/2} \cdot \gamma_l^{-1} - \pi_e \cdot \gamma_l^{-1} \qquad (1)$$

in which π_e denotes the surface free energy reduction of the solid due to adsorption from the gas phase surrounding the liquid droplet, generally referred to as the equilibrium spreading pressure.[15]

For XPS analysis the samples were mounted on gold coated sample holders with double-sided sticky tape and inserted into the spectrometer (Vacuum Generators

ESCA 3 Mk II equipped with a Tracor Northern TN 1710 signal averager for signal to noise ratio enhancement). Six samples could be inserted simultaneously in the machine, yielding a relatively high residual, but still acceptable, pressure in the spectrometer of approximately 10^{-8} torr due to continued outgassing of the enamel samples. A magnesium anode (1253.6 eV) was used for X-ray production (14 kV; 20 mA). After a scan of the overall spectrum at a low resolution (100 eV pass energy) peaks were recorded at a high resolution (50 eV pass energy) in the following order: C_{1s}, O_{1s}, N_{1s}, P_{2p}, Ca_{2p} and C_{1s} again (control). Occasionally Cl_{2p}, Na_{2s}, F_{1s} or $Sn_{3d5/2}$ peaks were recorded after Ca_{2p}, dependent on the type of surfactant applied.
The area under each peak was used for the calculation of peak intensities, while linearly correcting for the background contribution, from which the apparent elemental surface compositions (%) were obtained employing sensitivity factors determined by Wagner *et al.*[16] Exact binding energies of the peaks were calculated with respect to the C_{1s} peak set at 285.0 eV in order to correct for the charging of the samples. 5 pieces were measured per surface treatment.

3.4. RESULTS

In Table 3.2 the estimated surface free energies are shown for the various treatments together with contact angles measured with water and α-bromonaphthalene. The enamel surface free energy increased after application of all mouthrinses as well as after salivary protein adsorption, except for Meridol® which slightly reduced the enamel surface free energy. Application of the rinses to pellicle coated enamel yielded an increased surface free energy as compared to the surface free energy of pellicle coated enamel. Meridol®, however, again demonstrated a clear exception, yielding a reduced enamel surface free energy when applied on pellicle coated enamel.

Table 3.2. Estimated surface free energies γ_s ($mJ.m^{-2}$) and contact angles (degrees) measured with water and α-bromonaphthalene for variously treated enamel surfaces (± denotes S.D.) n = 5 specimens.

Treatment	γ_s	Θ_{H_2O}	$\Theta_{\alpha\text{-Br}}$
None	85 ± 5	43 ± 4	17 ± 3
+S	103 ± 4	48 ± 3	26 ± 6
+Pe	113 ± 4	33 ± 4	19 ± 2
+S+Pe	121 ± 1	28 ± 3	19 ± 2
+Pe+S	115 ± 3	38 ± 9	17 ± 3
+Md	82 ± 7	60 ± 7	26 ± 2
+S+Md	80 ± 4	60 ± 7	18 ± 3
+Md+S	92 ± 3	50 ± 9	20 ± 3
+L	103 ± 7	43 ± 9	20 ± 2
+S+L	116 ± 5	30 ± 4	18 ± 2
+L+S	105 ± 3	40 ± 5	20 ± 3
+V	119 ± 2	29 ± 3	17 ± 2
+S+V	100 ± 3	48 ± 3	22 ± 3
+V+S	109 ± 4	38 ± 3	19 ± 2
+Pr	114 ± 2	35 ± 5	17 ± 2
+S+Pr	109 ± 3	36 ± 3	18 ± 2
+Pr+S	108 ± 3	41 ± 3	18 ± 3
+Me	109 ± 3	39 ± 3	26 ± 4
+S+Me	112 ± 3	35 ± 4	22 ± 5
+Me+S	119 ± 3	28 ± 2	28 ± 6

All surface free energies converged to a value of approximately 108 ± 9 $mJ.m^{-2}$ if the variously treated enamel samples were covered with adsorbed salivary constituents. The variation within the contact angles for α-bromonaphthalene was small over the range of enamel surface treatments employed (20° ± 3°), giving rise to an almost identical dispersion surface free energy component (41-43 $mJ.m^{-2}$).

In Table 3.3 the elemental surface composition of human enamel with and without adsorbed salivary constituents after mouthrinse application are presented as expressed in a percentage over all elements detected. The standard deviations measured over 5 samples are approximately 5%, 5%, 15%, 10% and 20% for the Ca, C, O, N and P percentages, respectively.

Table 3.3. Apparent elemental surface composition (%) of human enamel with and without adsorbed salivary constituents after application of commercially available mouthrinses. ND denotes not detectable.

	Ca	C	O	N	P	Sn	F	Na
None	14.1	26.8	46.5	1.3	11.3			
+S	2.0	61.5	22.0	12.6	1.8			
+Pe	10.9	38.0	36.9	5.5	8.6			
+S+Pe	2.9	62.0	23.6	9.4	2.1			
+Pe+S	4.4	54.0	27.2	10.5	3.8			
+Md	4.0	60.4	24.8	2.8	3.6	1.6	2.4	
+S+Md	1.2	70.7	19.3	4.5	2.0	1.8	0.5	
+Md+S	1.6	65.5	21.5	10.1	1.3	0.5	0.2	
+L	11.9	33.6	42.6	3.9	8.5			
+S+L	7.9	41.7	35.4	8.9	6.1			
+L+S	6.7	46.2	32.8	8.9	6.4			
+V	8.8	41.7	39.1	2.6	7.6			
+S+V	2.9	63.1	27.4	3.9	2.7			
+V+S	4.4	50.2	29.3	11.6	4.5			
+Pr	11.6	33.5	41.6	0.3	8.2		1.0	3.4
+S+Pr	3.2	61.2	28.2	2.3	2.3		ND	2.6
+Pr+S	2.2	61.9	24.2	8.6	1.9		ND	0.9
+Me		63.0	28.5	2.2	3.0			
+S+Me		73.2	22.1	3.2	0.8			
+Me+S		67.2	20.7	9.5	1.1			

When the enamel had been treated with the rinses, the calcium and phosphorus percentages were reduced particularly after treatment with Meridol® or Merocet®, yielding a substantial screening of both calcium and phosphorus. A pronounced reduction in calcium and phosphorus percentages was also observed after adsorption of salivary constituents. In all cases, where saliva was applied on top of the enamel or on top of a mouthrinse, the percentage nitrogen increased.

3.5. DISCUSSION

In this study changes of surface free energy and elemental surface composition of human enamel, due to the adsorption of components from commercially available mouthrinses on enamel with and without an acquired pellicle, were determined by means of contact angle measurements and X-ray Photoelectron Spectroscopy.

The elemental surface composition obtained by XPS for polished enamel as presented in Table 3.3 can be used to calculate the Ca/P ratio, yielding Ca/P = 1.26. This is much lower than expected for pure stoichiometric calcium hydroxy-apatite (Ca/P = 1.67) or dental enamel (Ca/P = 1.4 - 1.6).[17] Equally low Ca/P ratios such as found in this study have been reported by Kuboki *et al.*[18] and Konishi *et al.*,[19] also using XPS. Hence, it remains unknown whether the observed Ca-deficiency of the outermost enamel surface is real, or due to the use of the Wagner sensitivity factors,[16] which may not be the most appropriate ones to use for dental enamel. However, O/P ratios measured (O/P = 4.7) closely match hydroxyapatite values. It should also be noted that the amount of C observed on ground and polished enamel is surprisingly high even if it is realised that a minor C contribution may arise from the organic matrix and possibly carbonaceous contaminants on the enamel surface. However Kuboki *et al.*[18] found a similarly high C content of the enamel surface, whereas furthermore the contact angles on our samples agree well with literature values.[20] Thus the possibility of inappropriate sample handling seems to be ruled out, leaving the origin of the C unknown.

Despite the fact that mouthrinses with widely different components were employed throughout this study, surprisingly little variation in surface free energy was found after application of the rinses to ground and polished enamel both with as well as without a pellicle. Only the application of Meridol® (based on aminefluoride and stannousfluoride) showed a reduction in enamel surface free energy which was more pronounced when applied on saliva coated enamel compared to uncoated enamel. De Jong *et al.*[21] also described a surface free energy reduction of enamel after 5 minutes application of aminefluoride (Elmex® fluid 0.1% F^-), whereas Glantz[22] reported a similar effect of stannousfluoride if applied either very long (8 days) or in extremely high concentrations (25000 ppm). Considering the concentration of aminefluoride (125 ppm F^-) and stannousfluoride (125 ppm F^-) in Meridol®, the surface active effects of the rinse observed are most likely caused by the aminefluoride. We measured significant surface active effects of Prodent®, which is based on sodiumfluoride. Previously De Jong *et al.*[23] found no such effects of sodiumfluoride applied alone on ground and polished enamel.

Most of the elemental surface compositions obtained by XPS of treated enamel do not match the elemental composition of the therapeutically active components of a product. This is partly due to the fact that screening of calcium, phosphorus and also oxygen from the enamel by adsorbed layers is incomplete and hence the apparent elemental surface compositions in Table 3.3 cannot be attributed entirely to the adsorbed layers.

From the fact that calcium and phosphorus signals are almost fully screened for Meridol® and Merocet® we can conclude that these rinses leave a thick coating; however the estimated thickness of the adsorbed layers is too high to be due to aminefluoride only. Therefore it may be expected that adsorption of other components than the therapeutically active ones may also have a considerable influence on both the elemental surface composition as well as on the enamel surface free energy. To verify this suggestion we determined (unpublished data) the surface free energy of enamel after 2 minutes application of sodiumlaurylsulfate, a

major additive to most commercial products. Sodiumlaurylsulphate (SLS) applied on ground and polished enamel yielded a very high surface free energy (130 $mJ.m^{-2}$). It can therefore be speculated that in many of the products investigated other additives (most likely SLS) cause an increase of the surface free energy, and a deviation of the surface composition from the one expected on basis of the molecular structure of the therapeutically active components.

An increase of the enamel surface free energy is an undesirable and thermodynamically unfavourable side effect, since several clinical studies[20,24] have shown that on low surface free energy materials, plaque accumulation is much smaller than on high surface free energy materials. *In vivo* experiments with chlorhexidine,[3] cetylpyridiniumchloride[6] or sanguinarine[5], however, all rinses showed a more or less reduced plaque accumulation, most likely indicative for a lower number of bacteria to adhere. This gives rise to the conclusion that the bactericidal effects of these mouthrinses completely overrule the unfavourable effects of an increased surface free energy which can be expected from a thermodynamical point of view.[15] As it is most likely that adsorption of SLS or other additives causes the unfavourable increase of the enamel surface free energy, the efficiency of a product will probably increase when these additives are not incorporated. Probably most products will than have a double working mechanism, based on enamel surface free energy reduction and based on anti-bacterial properties of the active components.

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CHAPTER 4

PHYSICO-CHEMICAL PROPERTIES OF COMMERCIALLY AVAILABLE MOUTHRINSES

4.1. ABSTRACT

This study evaluates physico-chemical properties of eight commercially available mouthrinses, viz their surface tension, *in vivo* enamel contact angle, viscosity, penetration coefficient, acidity and buffer capacity. The penetration coefficient, determined by the surface tension, contact angle and viscosity, is a measure for the ability of a liquid to penetrate into a capillary space, such as interproximal regions, gingival pockets and pores. The acidity is often determined by a compromise between requirements, set by taste, enamel de- and remineralisation and stability of the solution. Among the eight mouthrinses evaluated, their physico-chemical properties differed greatly. Particularly the penetration coefficient varied by a factor of 1.8 over the products tested, whereas surprisingly several products were extremely acidic.

4.2. INTRODUCTION

Mouthrinses are frequently used in dentistry to improve dental health. They can be useful in preventing plaque-accumulation by addition of anti-bacterial compounds such as chlorhexidine[1,2] or aminefluorides[3,4] or alternatively in stimulating enamel

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remineralisation by the addition of fluorides.[5,6] Current research on and evaluation of mouthrinses have focussed on the overall effectiveness of the products, for example their effects on plaque and enamel remineralisation. In order to be effective on smooth surfaces, a mouthrinse should spread completely over the tooth surface to enlarge its contact area and to form a zero contact angle. A mouthrinse should be able to penetrate well in order to be effective in interproximal spaces and gingival pockets. The capacity of a liquid to penetrate in pores and capillary spaces is determined by its viscosity, surface tension and contact angle on the capillary surface and is expressed by the penetration coefficient. A liquid with a low viscosity, a high surface tension and a low contact angle can penetrate fast into a capillary space.[7] Regarding the periodontal pocket as a capillary space, the penetration coefficient becomes an important factor to enhance the effect of a rinse in pockets.

Product formulation is often governed by a balance between anti-bacterial effects, de- and remineralisation effects, stability[8] and, last but not least, taste requirements. Sometimes these requirements are conflicting, resulting in undesirably acidic products.

It is the aim of this study to evaluate physico-chemical properties of eight commercially available mouthrinses, being: their surface tension, *in vivo* enamel contact angle, viscosity, penetration coefficient, acidity and buffer capacity.

4.3. MATERIALS AND METHODS

All mouthrinses used in this study were purchased commercially and used "as received" within 3 months. The following products were evaluated: Hibident® (containing 0.2 percent chlorhexidine, as given by the manufacturer, ICI, Belgium), Prodent® (containing 0.05 percent NaF, Intradal, Amersfoort, The Netherlands), Merocet® (containing 0.05% cetylpyridinium chloride, Merell Pharmaceuticals Ltd, UK), Meridol® (containing 125 ppm AmF and 125 ppm SnF_2, GABA, Basel, Switserland), Listerine® (containing various phenolic compounds, Lambert Chemical

Co. Eastleigh, UK), Veadent® (containing 0.03 percent sanguinarine, Vipont Lab. Inc. Ft., Collins, USA), Act® (containing 0.05 percent NaF, Johnson & Johnson, Benelux, Amersfoort/Brussel) and Oraldene® (containing 0.1 percent hexetidine, Warner Lambert Health Care, Eastleigh, UK).

The surface tension (γ_l , $mJ.m^{-2}$) was determined with a Lauda Autotensiomat equipped with a thermostat (Lauda, Königshafen; West Germany). The temperature was kept constant at 25°C. The surface tension was measured with a Du Nouy ring (circumference 0.06 m), which travelled with a velocity of 1.4×10^{-4} $m.s^{-1}$, pulling a thin liquid film out of the bulk fluid. The force needed to disrupt the film is measured and computed by the autotensiomat into the surface tension. Corrections were made for the weight of the film based on the Harkins Jordan factor.[9] The accuracy of the apparatus is 0.1 $mJ.m^{-2}$.

Contact angles *in vivo*: In order to determine the wetting properties of the rinses, the contact angle (Θ, degrees) between the tooth surface and a liquid droplet of the rinse was measured *in vivo*. Eight test persons participated in this study. They were seated in a dental chair in such way that the buccal surface of the central incisors was positioned horizontally. The test persons were requested not to drink tea or coffee two hours prior to the measurements.

After drying the surface with a vacuum suction tip during 60 s, a 1 μl droplet of the rinse was placed on the tooth surface avoiding those areas with visible plaque, and a colorslide was made.[10] The contact angle was subsequently calculated from the base width and height of the droplet measured on enlarged slides yielding an accuracy of 1 degree per reading.[11]

Viscosity: The viscosity (η, $kg.m^{-1}.s^{-1}$) of the products was measured with an Ubbelohde viscosimeter. The calibration was checked regularly by measuring the viscosity of water. This instrument is based on the principle of laminated flow through a cylindrical capillary under the influence of gravity. The flow time of a fixed volume of mouthrinses through the capillary is measured, yielding the viscosity according to:

$$\eta = c.t \qquad (1)$$

in which:

c = calibration constant as given by the manufacturer

t = flow time (seconds)

The flow time was recorded four times per filling, and two fillings were made for each mouthrinse, in order to obtain an accuracy of 0.0001 $kg.m^{-1}.s^{-1}$.

Penetration coefficient: The penetration coefficient (pc, $m.s^{-1}$) is calculated from the surface tension of the rinse, the contact angle of the rinse on the *in vivo* tooth surface and the viscosity, according to:[7]

$$pc = \gamma_1 \frac{\cos\theta}{2\eta} \qquad (2)$$

Acidity and buffer capacity: The pH of the rinses was measured with an A161 pH meter (Ankersmit, Belgium) with an accuracy of 0.1 pH unit. A combined glass electrode was used to determine the pH of 15 ml of the rinse at room temperature. The electrode was stabilized in a pH = 7.00 and a pH = 4.01 buffer solution. The pH was registered when the display was stable for 5 seconds.

The buffer capacity (ß, $mol.l^{-1}$) is defined as the number of moles of strong base or acid required to cause a unit increase or decrease in the pH of one liter liquid, and was measured by adding small amounts (0.1 ml) of either a 0.05 M KOH solution or a 0.05 M HCl solution to 15 ml of a mouthrinse until the pH had changed one unit, under constant stirring at 250 rpm.

4.4. RESULTS

The results of the various measurements are compiled in Table 4.1. The surface tensions of the rinses are all in the range between 30 $mJ.m^{-2}$ and 40 $mJ.m^{-2}$. Listerine® demonstrates the highest viscosity, Prodent® the lowest. *In vivo* contact

Table 4.1. Surface tension (γ_l), viscosity (η), *in vivo* contact angle (Θ), pH and buffer capacity (β_{KOH}, β_{HCl}) of the mouthrinses evaluated in this study.

	γ_l $mJ.m^{-2}$	$\eta^{(a)}$ $kg.m^{-1}.s^{-1}$	$\Theta^{(b)}$ degrees	pH	β_{KOH} $(mol.l^{-1})$	β_{HCl} $(mol.l^{-1})$
Hibident®	39.5	0.1069	53	6.2	$2.4x10^{-4}$	$5.7x10^{-4}$
Prodent®	35.0	0.1052	47	6.4	$6.1x10^{-3}$	$2.4x10^{-2}$
Merocet®	30.6	0.1471	41	5.9	$6.3x10^{-3}$	$1.6x10^{-2}$
Meridol®	32.7	0.1065	40	3.7	$3.1x10^{-3}$	$6.9x10^{-3}$
Listerine®	33.7	0.1738	49	4.3	$6.0x10^{-3}$	$6.2x10^{-3}$
Veadent®	32.1	0.1402	54	4.2	$2.9x10^{-3}$	$7.2x10^{-3}$
Act®	37.0	0.1524	42	7.5	$1.6x10^{-2}$	$3.3x10^{-2}$
Oraldene®	30.9	0.1158	37	3.6	$3.2x10^{-3}$	$8.1x10^{-3}$

(a) S.D. amounts 5 percent over four fillings of the viscosimeter.

(b) S.D. over 8 test persons amounts 20 percent on an anverage.

angles are between 37 and 54 degrees. Oraldene® and Meridol® are rather acidic products (pH 3.6 and 3.7 respectively). The buffer capacities are relatively high for Act®, Prodent® and Merocet®, whereas the buffer capacity of Hibident® is quite low. Fig. 4.1 illustrates the effects of adding KOH or HCl on the pH of Prodent® (high buffer capacity) and Hibident® (low buffer capacity). Surface tensions, viscosities and contact angles given in Table 4.1 have been used to calculate the penetration coefficients of the products summarized in Fig. 4.2. Analysis of the error propagation of surface tension, viscosity and contact angle shows that on the average a variability of 20 percent exists in the penetration coefficient calculated. Within this

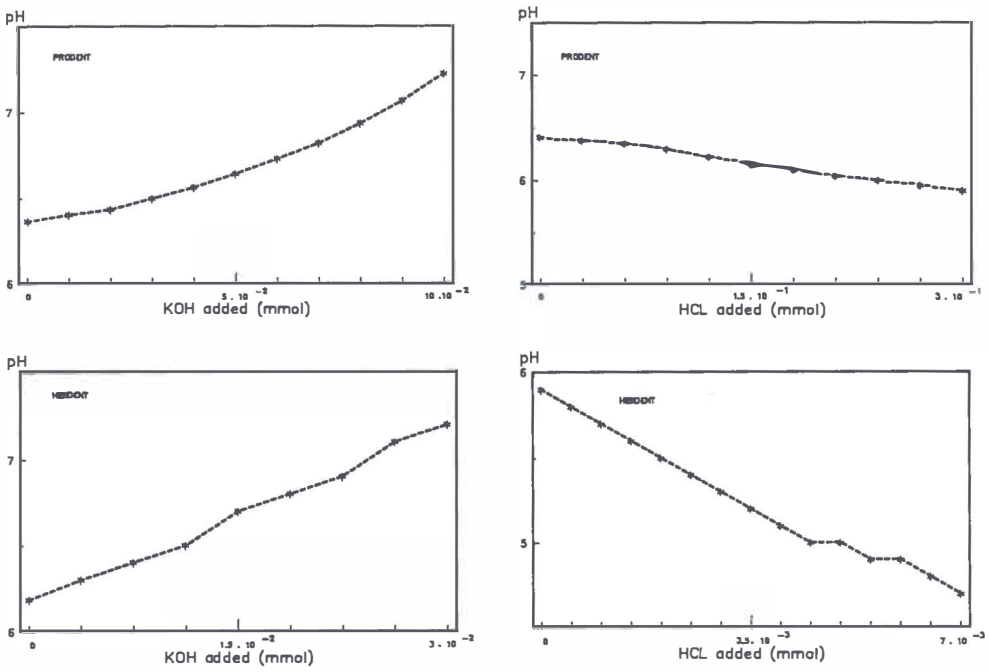

Fig. 4.1. Changes in pH of Prodent® and Hibident® upon addition of small amounts of KOH or HCl to 15 ml of the mouthrinse.

experimental variability, Hibident®, Prodent®, Meridol® and Oraldene® demonstrate the highest penetration coefficients, whereas the penetration coefficients of Listerine® and Veadent® are lowest.

4.5. DISCUSSION

In this study we evaluated some physico-chemical properties of commercially available mouthrinses. In the literature little attention is given to these properties, despite the fact that they can be important, particularly with regard to the penetration of the products in interproximal spaces and pores.

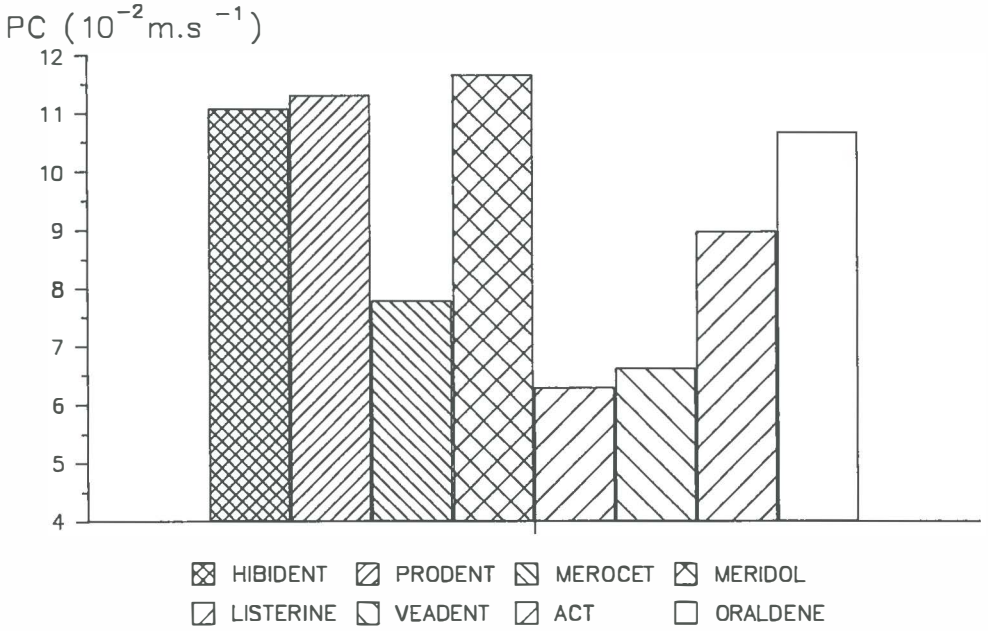

Fig. 4.2. Penetration coefficients (pc, $m.s^{-1}$) of the mouthrinses evaluated in this study, as calculated from the measured surface tension, *in vivo* contact angle and viscosity of the products (see Eq. (2)).

The surface tensions of the mouthrinses are all considerably lower than that of pure water[12] (72.2 $mJ.m^{-2}$) and that of saliva[13] (53.0 - 63.0 $mJ.m^{-2}$). Furthermore it can be noted that the surface tension of Meridol® the product based on aminefluoride/ stannousfluoride, is in the same range as observed for pure aminefluoride solutions.[14] All products are more viscous than water (0.1002, $kg.m^{-1}.s^{-1}$,).[12] The *in vivo* contact angles of the mouthrinses on tooth surfaces are all between 37 and 54 degrees, which is comparable to clinically registered contact angles of water on *in vivo* tooth surfaces.[15] Based on this *in vivo* water contact angle (48 $\pm$ 4 degrees) and assuming that the wettability of tooth surfaces is similar to the one of pocket surfaces, it can be calculated that the penetration coeffient of water in gingival sulci is extremely high and equal to 0.241 $m.s^{-1}$ (compare also Fig. 4.2) so that water will therefore

penetrate much better in a gingival sulcus than the mouthrinses evaluated in this study. Irrigation techniques are used more and more in dental practice to enhance gingival health. Newman and coworkers[16] observed that irrigation with water alone significantly reduced the gingival index (30 percent), gingival bleeding (44.9 percent) and bleeding upon probing (24.6 percent), but it did not affect the amount of plaque. Lander *et al.*[17] found similar results, showing that water might have a beneficial effect on the gingival health. This means that it would be worthwile to modify mouthrinse formulations in order to increase their penetration, although of course the capacity of a product to penetrate is only one of the many properties (taste, colour, bactericidal properties) determining the ultimate efficacy of a product.

It is difficult to understand the reason why some manufacturers tend to produce relatively acidic mouthrinse formulations. A possible reason could be that taste requirements are met more easily by a more acidic product or that acidic products are more stable.[8] A combination of SnF_2 and AmF in Meridol® for example is more stable at a low pH[18], while also a low pH might stimulate the formation of CaF_2, which is thought to form a "protective layer" on enamel from which F^- ions are slowly released.[19] Yet the combination of a low pH and a high buffer capacity seems undesirable and is likely to enhance enamel demineralisation.

In summary it can be stated that large differences exist between surface tension, *in vivo* contact angle, viscosity, penetration coefficient, acidity and buffer capacity of commercially available mouthrinses. On the basis of this study and available literature the expectation seems justified that the subgingival action of products can be stimulated by increasing their penetration ability.

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CHAPTER 5

A COMPARISON OF BACTERIAL GROWTH INHIBITING EFFECTS OF SIX COMMERCIALLY AVAILABLE MOUTHRINSES

5.1. ABSTRACT

In this study the bacterial growth inhibiting effects of six commercially available mouthrinses (Hibident®, Prodent®, Merocet®, Listerine®, Veadent® and Meridol®) were determined. Hibident® was used as a positive control. Five strains of oral bacteria were tested (*Streptococcus mutans* C67, *Streptococcus sanguis* CH3, *Veillonella alcalescens* V1, *Lactobacillus acidophilus* JP and *Actinomyces viscosus* C74), as representatives of the supragingival human microflora. The Maximal Inhibiting Dilution (MID) was measured in batch cultures for each product and strain. With respect to the positive control Hibident® containing 0.2% chlorhexidine), the most effective product was Meridol® (containing 125 ppm aminefluoride 297 and 125 ppm stannousfluoride) followed by Merocet® (containing 0.05% cetylpyridiniumchloride), Veadent® (containing 0.03% sanguinarine), Listerine® (containing phenolic compounds) and Prodent® (containing 0.05% sodiumfluoride). Although all products have been reported separately to yield a plaque reduction *in vivo*, this study provides a firm basis for a comparison between products, as they were all evaluated in the same way.

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5.2. INTRODUCTION

The presence of dental plaque on teeth has been known since Anthony van Leeuwenhoek gave the following description in 1863 "little animals in the white matter" on his teeth. Its key role in the development of caries and periodontal disease is evident.[1,2]

Dental plaque is described as a soft, predominantly bacterial material which develops on the surfaces of teeth and other oral surfaces.[3] *Streptococcus mutans*, lactobacilli and *Actinomyces viscosus* tend to colonize preferentially on the tooth surfaces.[4] It is a well accepted theory that especially these bacteria metabolize carbohydrates and other food components to acids which demineralize the enamel. Plaque removal is essential in preventing the development of carious lesions and gingival inflammation. Various methods are used to remove food particles, plaque and debris from the teeth such as toothbrushing, dental floss or toothpicks. In recent years mouthrinses containing anti-bacterial agents have been used to prevent plaque accumulation. Several compounds like antibiotics, enzymes, quaternary ammonium compounds, phenolic compounds or bis-biguanides are used.[5]

For several reasons antibiotics and enzymes are not appropriate for long term plaque control uptill now. Sensitization of the patient or opportunistic infections can develop, making the antibiotic useless for more severe infections. In general the working mechanism of enzymes is too specific to render a good anti-plaque effect.[6] Chlorhexidine is a desinfectant and is therefore able to prevent plaque accumulation but has some undesirable side effects such as loss of taste and discoloration of teeth.[7] Hence researchers try to develop other anti-bacterial compounds for mouthrinsing. Hitherto, no other compound has been found however, with the same plaque preventing properties as chlorhexidine, despite the fact that numerous products have appeared on the market. Most of these products have been evaluated in separate studies for their effects on plaque prevention and inhibition of bacterial growth.[8-13] It is impossible, however, to make a real comparison of products on the basis of these

studies due to differences in evaluation methods. Therefore, it is the aim of this study to compare the effects of six commercially available mouthrinses on the growth of five supragingival strains of oral bacteria which play an important role in the occurrence of dental caries and gingivitis.

5.3. MATERIALS AND METHODS

The mouthrinses employed in this study were all commercially obtained and used within three months after purchasing. Hibident®, a product based on chlorhexidine was included as a positive control. All products are listed in Table 5.1, together with their main additives and manufacturers.

Table 5.1. Commercially purchased mouthrinses evaluated in this study together with their main active components and manufacturer.

Tradename	Main active component	Manufacturer
Hibident®	0.2% chlorhexidine	ICI Dental Benelux Belgium
Prodent®	0.05% sodiumfluoride	Intradal The Netherlands
Meridol®	125 ppm aminefluoride 125 ppm stannousfluoride	GABA Basel Switzerland
Merocet®	0.05% cetylpyridinium chloride	Merell Dow Pharmaceutical United Kingdom
Listerine®	phenolic compounds	Lambert Chemical Co. Ltd. United Kingdom
Veadent®	0.03% sanguinarine	Vipont Laboratories Inc. Ft Collins, U.S.A.

The bacterial strains used in this study were selected to be representative for supragingival plaque and included *Streptococcus mutans* C67, *Streptococcus sanguis*

CH3, *Veillonella alcalescens* V1, *Lactobacillus acidophilus* JP and *Actinomyces viscosus* C74. These strains were originally isolated from supragingival plaque from the human oral cavity and fresh isolates were frozen in small aliquots in Todd Hewitt broth (OXOID) containing 7% (v/v) dimethylsulfoxide at -20°C. At the start of the experiment bacteria were grown overnight at 37°C in an appropriate broth, being Todd Hewitt broth (OXOID) for *S. mutans* C67 and *S. sanguis* CH3 and Brian Heart Infusion (OXOID) for *L. acidophilus* JP and *A. viscosus* C74. For *V. alcalescens* V1, Todd Hewitt broth was supplemented with 1% lactic acid. Anaerobic strains were cultured in an atmosphere of 80% CO_2, 15% N_2 and 5% H_2. These cultures, having densities between 0.5 x 10^9 and 1 x 10^9 cells.ml^{-1}, were used to inoculate second cultures containing various amounts of a mouthrinse.

Growth inhibiting effects of the mouthrinses for the various strains were first determined in a pilot study by adding widely varying amounts of a product to 10 ml bacterial culture. Thus different dilutions of the products were obtained and incubated again at 37°C for 18 hours. Subsequently the extinction of the test tubes was measured in a photospectrometer (Bausch & Lomb Spectronic) at a wavelength of 660 nm employing the broth as a control. These results were applied to make appropriate dilution series for each product and bacterial strain based on one bacterial culture. The average extinctions of two such dilution series were plotted as a function of the dilution factor. The dilution factor, at which the extinction was 10% below the control was taken as the Maximal Inhibiting Dilution (MID).

5.4. RESULTS

Fig. 5.1 shows examples for two products of the relation between the extinction and the dilution of a product. This figure includes two extreme examples; Hibident® inhibited growth up till approximately 2000 times dilution, whereas Prodent® only reduced growth in dilutions between 1 and 10. It should be noted that occasionally, normal variations in the extinctions of the control occurred, due to differences in

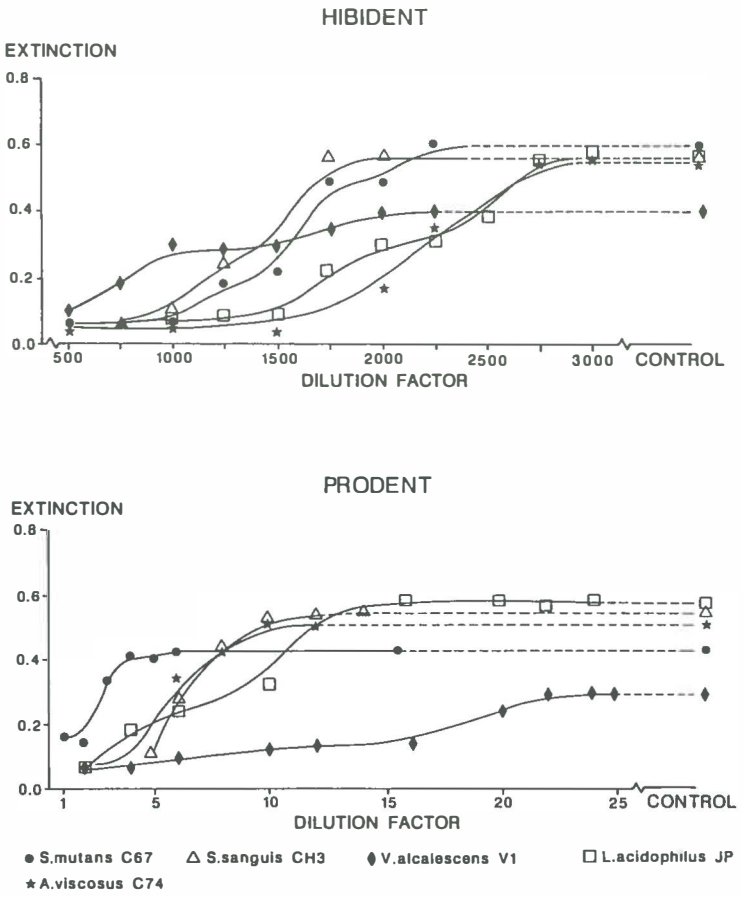

Fig. 5.1. Examples of the relations between the extinctions and the dilution factors of two products averaged over two separate dilution series. Data for the control, containing only the broth, are indicated at infinite dilution.

culture conditions. However, since each curve is based on one culture, this does not affect significantly the value for the MID's obtained. It can also be seen from this figure that certain strains were more sensitive to a given product than others. For example, *L. acidophilus* in the evaluation of Hibident® showed an extinction of the control of 0.6. The dilution factor at a 10% reduced extinction can be read from Fig. 5.1 to be approximately 2750. MID's thus determined are listed in Table 5.2 for all

Table 5.2. Maximal Inhibiting Dilution (MID) of six commercially available mouthrinses for various strains of oral bacteria obtained from results of two separate runs.

Strain	Hibident®	Prodent®	Meridol®	Merocet®	Listerine®	Veadent®
S. mutans C67	1:1750	1:6	1:1500	1:1000	1:12	1:60
S. sanguis CH3	1:1750	1:14	1:2000	1:875	1:36	1:70
V. alcalescens V1	1:2000	1:22	1:2000	1:500	1:50	1:100
L. acidophilus JP	1:2750	1:12	1:1750	1:625	1:90	1:50
A. viscosus C74	1:2750	1:10	1:2500	1:1500	1:30	1:30

strains and products. MID's derived from dilution series of separate bacterial cultures, differed never more than 4% from the averages of two cultures presented in Table 5.2.

Subsequently, we defined a relative effectiveness (RE) of a product according to:

$$RE = \frac{\text{MID product}}{\text{MID Hibident®}} * 100\% \qquad (1)$$

Fig. 5.2 shows the relative effectiveness of the products for the various strains. As can be seen, only Merocet® and Meridol® approached the effectiveness of Hibident®, whereas Prodent®, Listerine® and Veadent® were hardly effective in reducing bacterial growth in comparison with Hibident®. Because the effectiveness of the products differed per strain, an average effectiveness ($\widetilde{RE}$) was calculated by averaging the values of RE according to equation (1) over all strains involved, therewith obtaining a average relative effectiveness of the products ($\widetilde{RE}$) for the growth inhibiting effect on supragingival plaque. The $\widetilde{RE}$'s for all products are presented in Fig. 5.3.

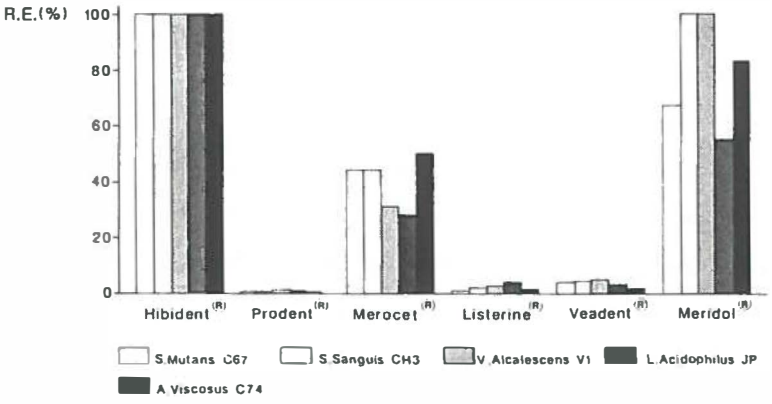

Fig. 5.2. The relative effectiveness (RE) in inhibiting bacterial growth of six commercially available mouthrinses on 5 bacterial strains (Hibident® is set at 100%).

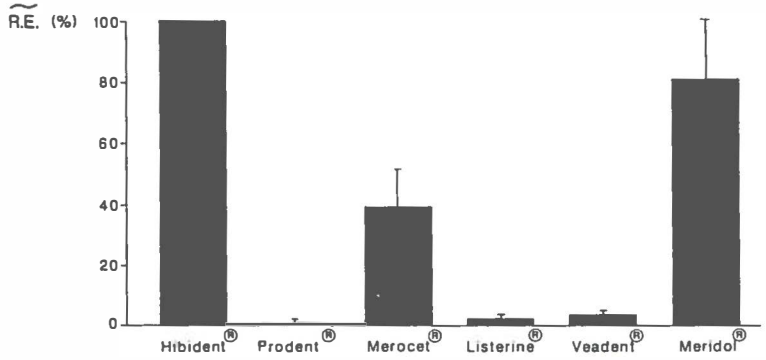

Fig. 5.3. The average relative effectivess ($\widetilde{RE}$) in inhibiting bacterial growth averaged over all strains involved of six commercially available mouthrinses on 5 bacterial strains (Hibident® is set at 100%).

5.5. DISCUSSION

In this study we compared the *in vitro* effects of six commercially available mouthrinses on the growth of five different supragingival bacterial strains. All effects were measured with respect to Hibident®, which was used as a positive control. Thus we defined a relative effectiveness RE, based on the ratio between the Maximal Inhibiting Dilution (MID) of a product and of Hibident®.

For the evaluation of commercially available whole products a MID seems more appropriate than the Minimal Inhibiting Concentration (MIC) usually employed for the evaluation of a given component.[14] MID and MIC are, however, not directly comparable quantities because flavouring agents, coloring agents and surfactants all present in commercial products can reduce the anti-bacterial properties of the active components.[15] Table 5.2 shows large differences between the sensitivities of the strains for a given product. The MID of Hibident® is extremely high for *L. acidophilus* JP, *A. viscosus* C74, *S. sanguis* CH3 and *S. mutans* C67. *V. alcalescens* V1

is relatively insensitive to Hibident®. The MID of Merocet® and of Meridol® approaches the value of Hibident®, although it should be noted that Meridol® is the only product significantly inhibiting the growth of *V. alcalescens* V1. Whereas Listerine® and Veadent® have a low MID for all strains, Prodent® hardly shows any growth inhibiting effects. Prodent®, however, is a product not designed to have anti-bacterial effects, its major component being sodiumfluoride, beneficial for enamel remineralisation and the prevention of demineralisation.

Schaeken *et al.*[16] determined the composition of human plaque in volunteers who were treated once with a chlorhexidine gel. They found that *S. mutans* and *A. viscosus* were strongly suppressed, but *S. sanguis* was much less affected, which is in accordance with our *in vitro* results as far as *A. viscosus* is concerned (see Table 5.2).

Although some authors[17] report a significant plaque reduction in volunteers employing Veadent®, our study points to a negligible bacterial growth inhibiting effect of Veadent® as compared to Hibident®. This is in correspondence with the clinical results of Abbas *et al.*[8] and Siegrist *et al.*[12] Fine *et al.*[18] reported also a plaque reducing effect for Listerine®, which turned out to be very small compared to a chlorhexidine product.[5] Clinically significant effects of Meridol® and Merocet® have been reported by Perdok *et al.*[11] and Llewelyn[9], respectively.

In only one of the studies mentioned above more than two products were simultaneously evaluated,[16] undoubtedly due to the big strain, extensive clinical trials put on human volunteers. Statistically significant plaque reductions of a product relative to a placebo, however, do not warrant any conclusions about the comparison with another product separately tested. All these factors contribute to the current controversies in the literature on the efficiency of diverse mouthrinses.

In general the plaque reducing effects of the various products predicted from this *in vitro* study (see Fig. 5.3), correspond with separate *in vivo* effects reported, despite the fact that the activity of agents *in vivo* can be greatly affected by e.g. a shorter duration of the action and the presence of saliva. As all products were evaluated here in an exactly identical way, a much firmer basis for comparison is provided,

yielding the conclusion that the bacterial growth inhibiting effect of Hibident® is the greatest, followed by Meridol® and Merocet®. Listerine®, Veadent® and Prodent® are of minor importance for inhibiting bacterial growth.

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CHAPTER 6

CLINICAL EFFECTS OF COMMERCIALLY AVAILABLE MOUTHRINSES ON THE DEVELOPMENT OF PLAQUE AND GINGIVITIS AND ENAMEL SURFACE FREE ENERGY

6.1. ABSTRACT

In this study we evaluated clinical effects of six commercially available mouthrinses on the development of plaque and gingivitis and on the tooth surface free energy *in vivo*. The following rinses were used: Hibident® (containing 0.2% chlorhexidine), Prodent® (containing 0.05% sodiumfluoride) Meridol® (containing 125 ppm aminefluoride, 125 ppm stannousfluoride), Merocet® (containing 0.05% cetylpyridinium chloride), Veadent® (containing 0.03% sanguinarine) and Listerine® (containing several phenolic compounds). Sixty test persons were selected for this study and requested to employ the same, non-fluoridated toothpaste during fourteen days. Both at day 0 as well as at day 14, the Plaque Index (PI), Gingival Index (GI), Planimetric Plaque index (PP) and the tooth surface free energy were assessed. After this preparatory phase, all oral hygiene was stopped for six days and only a rinse was used twice a day (30 seconds, 10 ml). After this period, all above mentioned parameters were measured again. In addition, the microbial composition of the plaque was determined at the beginning (day 0) and the end of the study (day 20). Hibident® (incorporated in this study as a positive control) demonstrated the lowest PI after 6 days use, whereas Meridol® demonstrated the lowest GI. PI and GI scores

This chapter has been accepted in Biofouling.

were highest after the use of Prodent® (incorporated as a negative control). None of the products were able to alter the tooth surface free energy markedly or to cause great shifts in microbial composition of the plaque. Since in this study six products were evaluated in a completely similar way, the results may provide a rigorous basis for comparing their clinical efficacies.

6.2. INTRODUCTION

The two most widespread diseases in mankind are dental caries and gingival inflammation, leading to the loss of teeth. Both diseases are initiated from dental plaque adhering to tooth surfaces. Dental plaque consists mainly of bacteria; other components are proteins, carbohydrates, ions, food debris and tissue cells. Bacteria present in the plaque ferment carbohydrates from food and beverages resulting in acid production.[1] These acids cause demineralisation of the outer enamel layer of teeth.

In principle, the initial state of this process is reversible. However, when the outer enamel layer (approximately 2 mm thick) is disrupted and the acids attack the inner dentin, the caries process becomes irreversible.[2] Further increase of acid attacks results in damaging of the pulp, inflammation and the loss of teeth.

The development of gingivitis is initiated in a similar way. Accumulated dental plaque causes inflammation of the gingiva, which is called gingivitis. One of the most recognizable symptoms of gingivitis is bleeding of the gingiva during toothbrushing. Gingivitis can develop into periodontitis, a more severe process, which ultimately leeds to the loss of teeth.[3]

Adhesion of indigeneous oral bacteria is an essential step in the development of dental plaque. The physico-chemical properties of the outer enamel surface are important for the initial adhesion of bacteria. Glantz as well as Quirynen *et al.* demonstrated that also under *in vivo* conditions, low surface free energy substrata collect less plaque than high surface free energy substrata.[4,5] Thus a possible pathway

of preventing or reducing plaque formation would be to coat the enamel surface or at least caries susceptible areas with a surface free energy reducing agent. One of the components currently in use in dentistry to this end is aminefluoride. Applied to ground and polished enamel (surface free energy = 84 ± 12 $mJ.m^{-2}$) it reduces the enamel surface free energy to 53 $mJ.m^{-2}$, which is on thermodynamic grounds believed to discourage bacterial adhesion.[6,7]

Classical oral hygiene programs are based on regular toothbrushing, the use of dental floss and toothpicks. A number of commercially available mouthrinses, containing anti-bacterial compounds, are claimed to have plaque reducing effects. Chlorhexidine is known as a very effective anti-plaque compound.[8-10] Chlorhexidine can bind to the bacterial cell wall, cause leakage of intercellular compounds and eventually cause cell death. An important aspect of the anti-plaque effect of chlorhexidine is that it binds to negatively charged groups in molecules covering teeth, tongue and mucosa. Chlorhexidine can then be subsequently slowly released in inhibitory concentrations, thus influencing bacterial growth for a prolonged period.[11-13]

Other anti-bacterial compounds with potential anti-plaque effects are hexetidine, cetylpyridinium chloride, sanguinarine and phenolic compounds. Fluoride containing mouthrinses are basically designed to enhance enamel remineralisation, but aminefluorides also possess anti-plaque properties.[6,14]

Numerous studies exist evaluating the beneficial effects of mouthrinses with respect to a placebo or chlorhexidine. No product has been found hitherto as effective as chlorhexidine. Most products can be shown however to be effective compared to a placebo. Relatively few studies[15,16] exist, however, in which more than two mouthrinses are evaluated simultaneously.

It is the aim of this investigation to evaluate the short term clinical effects of six commercially available mouthrinses on the development of plaque and gingivitis. As a secondary aim, changes in tooth surface free energies due to mouthrinse use are evaluated *in vivo*.

6.3. MATERIALS AND METHODS

6.3.1. Mouthrinses

The mouthrinses employed in this study were all commercially obtained and were used full strength according to recommendations by the manufacturer. The products are listed in Table 6.1, together with their main additives and manufacturer.

Table 6.1. Mouthrinses used in this study, together with their main additives and manufacturer.

Tradename	Main additive	Manufacturer
Hibident®	0.2% chlorhexidine	ICI, Dental Benelux, Belgium
Prodent®	0.05% sodiumfluoride	Intradal, Amersfoort The Netherlands
Meridol®	125 ppm aminefluoride 125 ppm stannousfluoride	GABA, Basel, Switzerland
Merocet®	0.05% cetylpyridinium chloride	Merell Dow Pharmaceuticals, UK
Listerine®	phenolic compounds	Lambert Chemical Co. Ltd, UK
Veadent®	0.03% sanguinarine	Vipont. Lab. Inc. Ft Collins, USA

6.3.2. Experimental design

Test persons. A test group of 60 people (31 males, 29 females, average age 29.1 ± 8.0 years) was selected from a dental practice in the countryside of Groningen. All persons considered themselves healthy, were free of carious lesions and demonstrated no signs of periodontal breakdown. None of the testpersons were using antibiotics or other medications. No female volunteers were reported being pregnant.
Clinical setup. At the beginning of the experiment (day 0) all clinical parameters including Plaque Index (PI)[17], Gingival Index (GI)[17] and the Planimetric Plaque Index (PP)[18] as well as the tooth surface free energy and microbial composition of the plaque were assessed by a professional dentist. Following this baseline examination all test persons were requested to brush their teeth with a non-fluoridated toothpaste (Prodent Rood, Intradal, Amersfoort, The Netherlands) for 14 days. They received no special oral hygiene instruction in order to let the study mimic normal conditions as much as possible. After this period (day 14) all clinical parameters, except the microbial composition, were recorded again. The test persons received at random a bottle of a mouthrinse and were requested to abandon all oral hygiene for 6 days. Test persons were instructed to use the rinse undiluted and to rinse twice a day for 30 seconds with 10 ml. After these 6 days all clinical parameters were assessed again (day 20). On day 0 and day 20, standardized plaque samples were taken with a sterile tip of a pocket probe from the buccal surface of the upper first right molar to determine the microbial composition of the plaque. The participants were requested not to drink tea or coffee one hour before the measurements.

6.3.3. Clinical assessment of plaque and gingivitis scores

The amount of plaque on the teeth was assessed according to the Plaque Index (PI) system.[17] The plaque on the four gingival sites of a tooth (buccal, mesial, lingual/

Table 6.2. Criteria used to assess the Plaque Index.[17]

Score	Criteria
0	No plaque present in the gingival area.
1	A film of plaque adhering to the gingival margin and adjacent area of the tooth. The plaque may only be recognized by running a probe across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket on the gingival margin and/ or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

palatinal and distal) is given a score from 0-3 (see Table 6.2). The PI for a tooth can be obtained by adding the scores and dividing by four. The total PI is obtained in the same way by adding all scores and dividing the sum by the total of surfaces involved.

The gingival condition was assessed according to the Gingival Index (GI) system.[17] The GI does not consider pocket depth, bone loss or other changes at the periodontium, but the criteria are confined to qualitative changes of the soft tissues (see Table 6.3). The total GI is assessed similarly to the PI score and varies between 0 and 3.

Table 6.3. Criteria used to assess the Gingival Index.[17]

Score	Criteria
0	Normal healthy gingiva.
1	Mild inflammation - slight change in color, slight oedema, no bleeding upon probing.
2	Moderate inflammation - redness, oedema, glazing, bleeding upon probing.
3	Severe inflammation - marked redness, oedema, ulceration tendency to spontaneous bleeding.

The Planimetric Plaque Index (PP) was assessed after staining the plaque present on the buccal surfaces of the upper and lower surfaces with a disclosing solution (En-de-Kay). Subsequently, a colourslide (Agfachrome 100 S) was taken with a clinical camera (Pentax Super A, equipped with a 100 mm Dental Macro lens). In order to improve reproducibility of the colourslides, the test person was seated in a dental chair equipped with an adjustable headrest. A pointer was placed at the porion (a fixed point at the skull in the ear) and the height of the headrest was recorded. The camera was placed horizontally in such way that the middle of both arches were in the middle of the view-finder of the camera. Afterwards the slides were projected on a digitizing tablet (Apple Graphics). The buccal outline of the incisors and the outline of the plaque were traced with a cursor. A computer program calculated the area of the tooth and the plaque. The PP was only calculated for the incisors in order to minimize errors due to angular distortion. The PP was defined as:

$$PP = \frac{\text{total plaque area}}{\text{total tooth area}} * 100\% \quad (1)$$

6.3.4. Determination of the microbial composition of the plaque

A standardized amount, as judged by eye, of supragingival plaque at the gingival margin of the upper right molar was sampled with the tip of a pocket probe and analysed for the presence of the following bacterial strains: total streptococci, *Streptococcus mutans*, veillonella, lactobacilli and actynomyces. These strains are, amongst others, considered to be responsible for the development of caries and gingivitis. The plaque sample was suspended in 1 ml of a sterile transport fluid,[19] and within two hours dispersed by sonication for 10 seconds at 70 watt using a microprobe (Braun Labsonic 1510) and subsequently serially diluted. Appropiate dilutions were placed on the following selective and non selective media: MSB agar (Difco) for the enumeration of *S. mutans*, Rogosa agar (Difco) for lactobacilli, veillonella agar, consisting of trypton, yeast extract, agar, and supplemented with salts, 5% defibrinated sheep blood, 1% lactic acid and 7.5 μg/ml vancomycin, and CFAT agar, and ETSA agar[20] was used for total anaerobic counts. Anaerobic incubations were performed in a jar containing cold palladium catalyst under an atmosphere of N_2, H_2 and CO_2 (85:10:5). Plates to be used were previously incubated in the same gas mixture for several hours. The effect of a rinse on microbial counts was compared with the results of Hibident® taken as a positive control according to:

$$\text{Relative anti-microbial effectiveness} = \frac{\frac{M_0}{M_{20}}}{\frac{H_0}{H_{20}}} * 100\% \quad (2)$$

in which:

- M_0 and M_{20} represent the percentage of microorganisms of a given strain at day 0 and at day 20 respectively, after the use of a mouthrinse.
- H_0 and H_{20} represent the percentage of microorganisms of the same strain at day 0 and day 20 respectively, after the use of Hibident®.

According to this scoring system, an effectiveness less than 100% denotes that the mouthrinse is less inhibitory against a microbial strain under study than is Hibident®. Contrary, an effectiveness greater than 100% denotes that the mouthrinse is more effective than Hibident®.

6.3.5. Contact angle measurements and surface free energy estimation

For the clinical registration of contact angles, the dental chair was tilted in such a way that the labial surfaces of the upper incisors of the test persons were in a horizontally position. In order to rapidly evaporate the loosely bound water off the teeth, necessary to prevent immediate complete spreading of the liquid droplets over the tooth surfaces, the surfaces were dried for 60 seconds in an air stream created by holding a dental vacuum suction tip approximately 5 mm above the tooth surface.[21] Small liquid droplets of 1 - 2 μl were placed at the tooth surface and a colourslide was taken, from which the height and base-width of the droplets were measured yielding the contact angle Θ according to:

$$\Theta = 2 \tan^{-1} \frac{2h}{b} \qquad (3)$$

in which:

h is the height of the droplet

b is the base-width of the droplet.

Care was taken not to wet the same spot twice or to place the liquid droplet on visible plaque. The contact angles were measured with water, water/n-propanol mixtures and α-bromonaphthalene. Subsequently the surface free energy was estimated by inserting the measured contact angles in the geometric mean equation while accounting for spreading pressures.[22-24]

$$\cos \Theta = -1 + 2 \frac{(\gamma_s^d \cdot \gamma_l^d)^{1/2}}{\gamma_l} + 2 \frac{(\gamma_s^p \cdot \gamma_l^p)^{1/2}}{\gamma_l} - \frac{\pi_e}{\gamma_l} \qquad (4)$$

where γ_l^d and γ_l^p are the dispersion and polar components of the liquid surface tension (γ_l) and $\pi_e = \gamma_s - \gamma_{sv}$ is the spreading pressure. γ_{sv} denotes the surface free energy of a solid with adsorbed vapor molecules, whereas γ_s is the surface free energy of the bare solid.

The solid surface free energy (γ_s) is calculated from:

$$\gamma_s = \gamma_s^d + \gamma_s^p \qquad (5)$$

in which γ_s^d is the dispersion surface free energy and γ_s^p is the polar surface free energy.

6.3.6. Statistical analysis

All statistical analyses were carried out with SPSS/PC$^+$ program. First, a Bartlett-box homogeneity test was done, on the six groups at baseline (day 0), in order to check whether there were any significant differences present prior to the use of the mouthrinses. The Bartlett-box homogeneity test was repeated for the groups on day 14. Multiple Analysis of Variance (MANOVA) was applied to determine statistically significant differences in efficacies of the mouthrinses, assuming a significance level $p < 0.05$.

6.4. RESULTS

6.4.1. Plaque and Gingival Indices

At day 0, the mean PI and GI scores over the entire test group of 60 people amounted 0.90 ± 0.38 and 0.38 ± 0.32, respectively. The Bartlett-box homogeneity test indicated that, at the onset of the study, these scores were not significantly different between the groups composed for the evaluation of a given product.
Evaluation of these scores after the preparatory phase, at day 14, showed that the average PI and GI scores had all significantly decreased to 0.72 ± 0.39 and 0.31 ± 0.29, respectively. Again, also at day 14, there was no significant difference for these scores amongst the individual groups. Thus it is likely to assume that all test persons

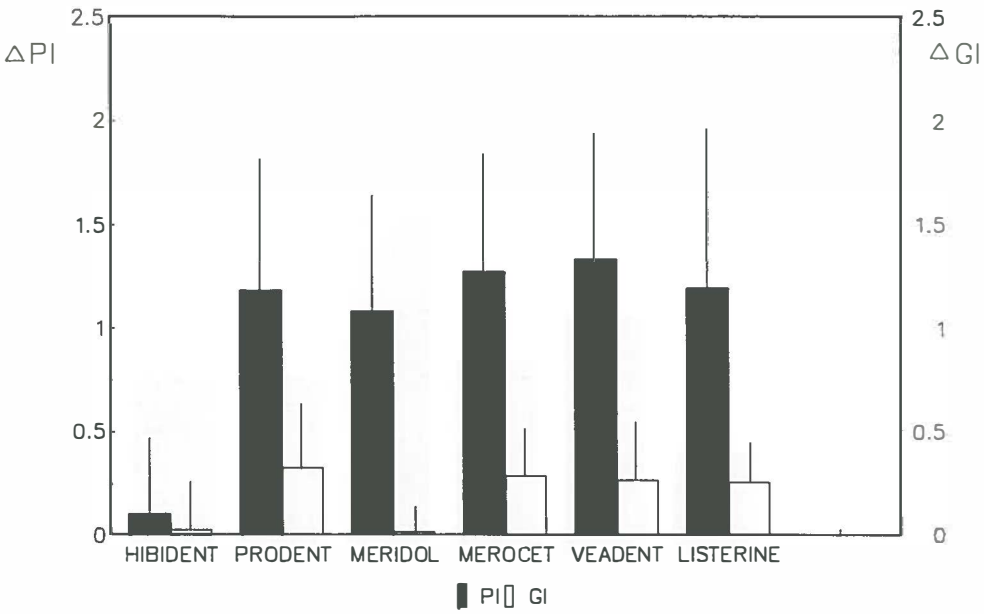

Fig. 6.1. Changes in Plaque Index (PI) and Gingival Index (GI) after six days use of a mouthrinse. The bars denote the S.D. over each test group (n=10).

adapted a better oral hygiene while participating in the study, despite the fact that no special instructions were given. Fig. 6.1 shows the changes in PI and GI scores, as developed from day 14 to day 20, i.e. during the use of a mouthrinse only. All products yielded significantly higher plaque and gingivitis scores at day 20 (after the use of the rinses) than at day 14, albeit with significant differences amongst the scores. MANOVA indicated a significant difference in ΔPI for Hibident® as compared to all other products. No significant difference in ΔPI was revealed for the other products. Furthermore, it was found that only Prodent® and Merocet® gave significantly higher ΔGI values than Hibident® and can thus be considered as non-effective in maintaining low GI scores.

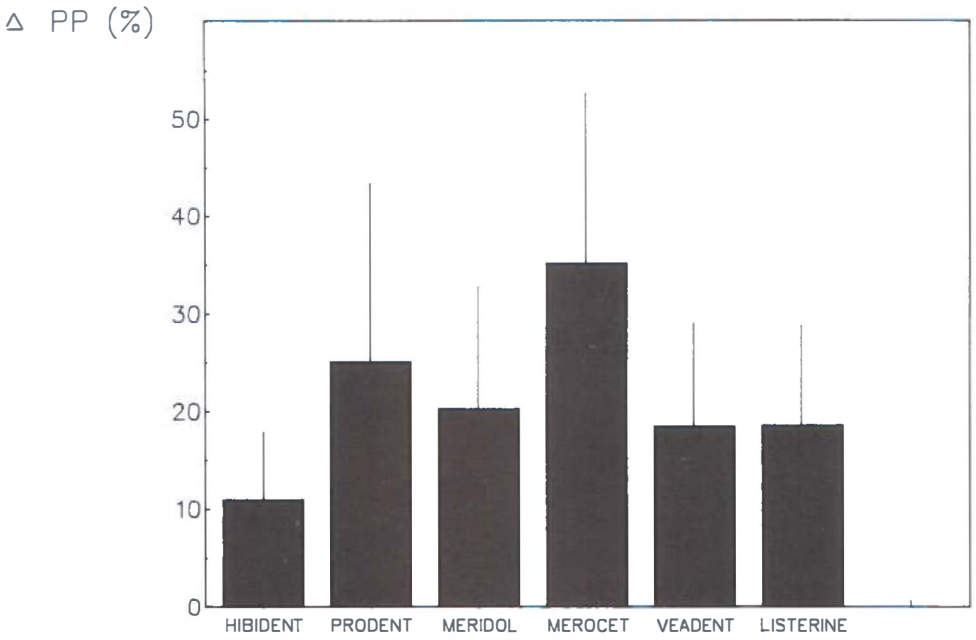

Fig. 6.2. Changes in Planimetric Plaque Index (PP) after six days use of a mouthrinse. The bar denotes the S.D. over each test group (n = 10).

6.4.2. Planimetric Plaque Index

At day 0, the PP score, averaged over the whole group was 6% ± 10%, with again no significant difference between the groups obtained after subdivision. Similarly, as described above for PI and GI, the PP score decreased during the preparatory phase to 4% ± 9%. Fig. 6.2 shows the change in PP scores between day 14 and day 20. MANOVA indicated no significant difference between these scores for Prodent®, Meridol®, Merocet®, Veadent® and Listerine®. Hibident® however, gave significantly better results.

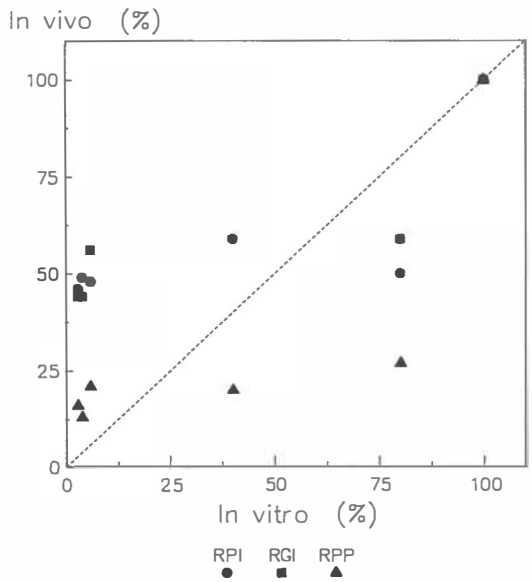

Fig. 6.3. A comparison between the *in vivo* and *in vitro* effectiveness of commercially available mouthrinses. A relative *in vivo* effectiveness was defined with respect to Hibident® for PI, GI and PP (Eqs. 7-9), whereas the *in vitro* effectiveness was defined according to Eq. (6) (see Perdok *et al.*[28] for details).
The dotted line represents the line of identity.

6.4.3. Microbial counts

The effect of the use of a mouthrinse on the microbial composition of the plaque is given in Fig. 6.4, expressed relative to Hibident® according to Eq. (2). Results for lactobacilli could not be given because the number of countable lactobacilli was too low to give reliable results. Hibident® is the most effective product with respect to *S. mutans*, actinomyces and veillonella. Considering total streptococci however, it appears that all the other products gave more favourable counts than Hibident®. Overall, there were no clear shifts in the microbial composition of the plaque after 6 days use of the rinses.

6.4.4. Contact angles and tooth surface free energy estimation

A selection of clinically registered contact angles for water and α-bromonaphthalene and the estimated surface free energies are listed in Table 6.4. None of the products was able to alter the tooth surface free energy drastically after 6 days use, whereas furthermore the use of a non-fluoridated standard toothpaste did not greatly influence the tooth surface free energy. However, within each of the separate groups, the standard deviation in the tooth surface free energy decreased markedly due to the use of a standard toothpaste (from 20 to 14 $mJ.m^{-2}$ on an average).

6.5. DISCUSSION

In this study we evaluated the effects of 6 commercially available mouthrinses on plaque and gingival indices, changes in the microbial composition of the plaque and on the enamel surface free energy. All products were evaluated with respect to each other and no placebo rinse was used. This is considered to be a particularly important point, since only a few studies exist in the literature in which more than three products are compared to each other.[25] Furthermore, it is emphasized that this

Table 6.4. Clinically registered contact angles for water and α-bromonaphthalene* and estimated tooth surface free energy at the onset of the study (day 0), after the preparatory phase (day 14) and after six days use of a mouthrinse (day 20) ± denotes S.D. (n = 10 for a product, n = 60 for the whole group)

Mouthrinse	Day 0			Day 14			Day 20		
	γ_s	Θ_{H_2O}	$\Theta_{\alpha\text{-Br}}$	γ_s	Θ_{H_2O}	$\Theta_{\alpha\text{-Br}}$	γ_s	Θ_{H_2O}	$\Theta_{\alpha\text{-Br}}$
Hibident®	85 ± 36	44 ± 20	25 ± 5	85 ± 15	62 ± 11	28 ± 3	92 ± 21	57 ± 16	24 ± 6
Prodent®	100 ± 14	50 ± 11	26 ± 6	87 ± 11	60 ± 11	22 ± 7	89 ± 14	55 ± 10	28 ± 4
Meridol®	82 ± 15	62 ± 13	27 ± 8	90 ± 14	47 ± 17	27 ± 7	96 ± 9	51 ± 9	27 ± 8
Merocet®	83 ± 18	61 ± 13	25 ± 5	83 ± 13	66 ± 11	25 ± 9	83 ± 20	61 ± 12	26 ± 10
Veadent®	86 ± 17	61 ± 11	31 ± 6	80 ± 15	68 ± 15	25 ± 7	90 ± 12	51 ± 10	25 ± 9
Listerine®	88 ± 20	55 ± 12	28 ± 7	83 ± 13	55 ± 14	37 ± 6	86 ± 11	59 ± 12	24 ± 5
Whole group	87 ± 20	56 ± 13	27 ± 6	85 ± 13	61 ± 14	26 ± 6	-	-	-

* data for water/n-propanol mixtures are not presented.

study was carried out in a representative test group and not in a community of dental students as often done.[6,26] Previously we have noticed that dental students, although very cooperative, usually demonstrate extremely low plaque and gingival scores and very low *S. mutans* counts[6] compared to a population with a smaller dental awareness.

6.5.1. Comparison of the clinical effects of the rinses

On the basis of this study, we can now compare the six products evaluated according to their clinical effectiveness with regard to plaque and gingivitis. Hibident®, a very effective rinse containing chlorhexidine (0.2%), is used as a positive control. Therefore, it is no surprise that this product turned out to be the best. Meridol® is less effective in reducing the amount of plaque, but it has beneficial effects on the gingiva. The GI is even slightly lower after 6 days use than after six days use of Hibident®. Another effect of Meridol® is the point shaped bleeding of the gingiva observed after probing. The effects of Listerine®, Veadent® and Merocet® are comparable. These effects are minor compared to Hibident® despite the claims of the manufacturers which are generally based on the effects of placebo related studies. The effect of Prodent® on PI and GI is negligible. We used this rinse as a negative control because it does not contain any specific anti-bacterial compound and the product was designed mainly to possess beneficial effects on de- and remineralisation of the enamel.[27]

6.5.2. Comparison of *in vivo* and *in vitro* evaluation of the rinses

In a previous study we evaluated the bacterial growth inhibiting effects of the same collection of mouthrinses as used in this study.[28] For each product a maximal dilution was determined and a relative effectiveness (RE) defined with respect to Hibident® according to:

$$RE = \frac{\text{Effect of a product}}{\text{Effect of Hibident®}} * 100\% \quad (6)$$

Similar to the above *in vitro* relative effectiveness we can define a relative effectiveness for the PI, GI and PP according to:

$$RPI = \frac{PI_{Product}}{PI_{Hibident®}} * 100\% \quad (7)$$

$$RGI = \frac{GI_{Product}}{GI_{Hibident®}} * 100\% \quad (8)$$

$$RPP = \frac{PP_{Product}}{PP_{Hibident®}} * 100\% \quad (9)$$

Fig. 6.3 compares the *in vitro* and *in vivo* effectivenesses and shows that no prediction can be made about the *in vivo* effect of a product on the basis of *in vitro* growth inhibition experiments.[29] The most important reason for this is probably that an *in vitro* study does not account for the "substantivity" of a product, i.e. the prolonged association between active components and oral surfaces.[13] Thus a reservoir can be build up and slow desorption of active components ensure that the minimal concentration required to inhibit bacterial growth remains available for a longer time in the oral cavity. Many products are efficient in inhibiting bacterial growth *in vitro*, but fail as a product once tested *in vivo* due to lack of sufficient substantivity[30], as can also be seen in Fig. 6.3.

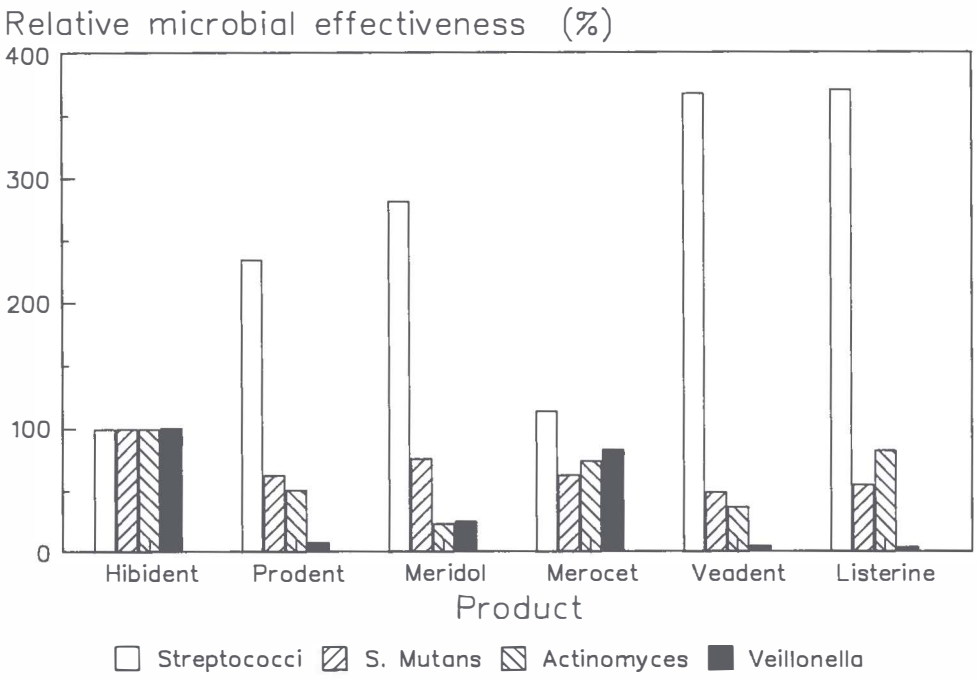

Fig. 6.4. Effects of the use of a given mouthrinse on the microbial composition of the plaque, expressed as compared to the effect of Hibident®, taken as a positive control (see Eq.(2)) Lactobacilli counts were too low to give reliable results.

6.5.3. Microbial effects of the rinses

The plaque composition was only evaluated for those strains which are thought to be responsible for the development of caries and gingivitis, e.g. *S. mutans*[31] veillonella[32,33], lactobacilli and actinomyces.[34] Although no clear shifts were observed in the plaque composition after only six days use of the products, Fig. 6.4 shows that Hibident® suppresses most effectively the presence of *S. mutans*, actinomyces and veillonella, but not of total streptococci. Total streptococci were clearly less abundant

in plaques that had developed during the use of the other products. This is most likely not a direct effect of the products but due to the fact that streptococci are effectively suppressed by growth of other surviving strains.

6.5.4. Effects on enamel surface physico-chemistry

None of the products was able to significantly alter the tooth surface free energy. Even Meridol®, the product based on aminefluoride which greatly decreases the surface free energy of ground and polished enamel, left the tooth surface free energy unaltered. This is largely in agreement with results from an *in vitro* study in which we applied the products either to ground and polished enamel or to saliva coated enamel. Yet, X-ray Photoelectron Spectroscopy (XPS) indicated that the elemental surface composition had greatly changed. Therefore we concluded that many other components from the products[35] had adsorbed, affecting the tooth surface free energy. We have also determined by XPS the thickness of the adsorbed layers[36] and found that only Merocet® and Meridol® left rather thick (5.6 - 6.7 nm) layers on the tooth surface. Thus major substantivity of these products could be expected as compared to Hibident®, leaving only a thin layer (3.7 nm). It is therefore hypothesized that cetylpyridinium chloride and aminefluoride may adsorb so strongly to the tooth surface that subsequent slow release necessary to maintain sufficient levels in the oral cavity does not occur.

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CHAPTER 7

GENERAL DISCUSSION

In this study we have made a comparison of the physico-chemical properties as well as of the clinical efficacy of commercially available mouthrinses. During the course of this study, another group of products have become available on the market, called 'pre-brushing rinses', which are thought to increase the amount of plaque removed by brushing. In this study, this type of products is not considered and only rinses which have more or less anti-bacterial properties are used. The fluoride containing rinse was employed as a negative control.

In this chapter we will consider the following aspects of mouthrinses:

1 - comparison of the clinical efficacies of the various rinses evaluated on the basis of this study and literature data,

2 - possible working mechanisms of the mouthrinses based on physico-chemical effects,

3 - future developments.

Ad. 1:- comparison of the clinical efficacies of the various rinses evaluated on the basis of this study and literature data.

As observed in numerous other studies, also this work shows that a rinse containing chlorhexidine is superior in creating low plaque levels in the oral cavity. Unfortunately, several disadvantages make chlorhexidine containing rinses not suitable for long term use. Table 7.1 gives a selection of studies published on commercially available mouthrinses between 1972 and 1990. These studies were selected because they pertain to rinses with active ingredients also used in our study and because they illustrate controversies in results between various research groups.

Table 7.1. A selection of studies in which commercially available mouthrinses are evaluated in order to illustrate the discrepancies in the conclusions of various studies (the numbers in the first column refer to the numbers in the references).

Study Ref.nr	Active compound	Placebo	Duration of study	Results
1	Chx NaF AmF/SnF_2 Cpc Phen Sang	no	6 days	Chx shows the lowest PI and GI values. AmF/SnF_2 is less effective; reasonable effect on PI and GI. The other compounds have minor effects.
2	Chx	yes	4 months	84% plaque reduction and 43% gingival inflammation reduction.
3	Chx	yes	3 months	0.12% Chx solution is as good as a 0.2% Chx solution in reducing plaque.
4	Chx	yes	6 months	The Chx rinse shows significant reduction of gingivitis, gingival bleeding and plaque accumulation compared to placebo.
5	Chx Sang	no	19 days	Sang has a limited role in plaque inhibition compared to Chx.
6	Chx AmF/SnF_2 Phen	yes	21 days	Chx is superior in plaque reduction compared to AmF/SnF_2 and Phen compounds.
7	AmF/SnF_2	yes	7 days	AmF/SnF_2 rinse demonstrates a significant lower PI and GI compared to a placebo.
8	Cpc DBr	water	3 months	A 30-58% gingivitis reduction is reported.
9	Cpc	no	12 days	Cpc does not inhibit plaque and gingivitis more than a toothpaste.

10	Phen List	yes water	9 months	55% reduction in plaque dry weight.
11	Sang	Chx no	5 days	Chx demonstrate significantly lower plaque scores than Sang.
12	Sang	yes	70 days	79% improvement in plaque scores with respect to placebo.
13	Sang	yes	5 days	no effect on sucrose stimulated plaque growth.
14	Sang Chx	yes	18 days	Chx possesses good anti-plaque properties, the effect of Sang is low.

Abbreviations:

Chx: chlorhexidine, NaF: sodiumfluoride, AmF: Aminefluoride, SnF_2: Stannous-fluoride, Cpc: Cetylpyridinium chloride, Phen: Phenolic compounds, Sang: Sanguinarine, DBr: Domiphen bromide, List: Listerine®.

From Table 7.1 we conclude that:

1. presently chlorhexidine containing rinses are superior in efficacy as compared to all other rinses in creating low plaque levels,
2. most studies compare only one rinse with respect to a placebo rinse or water,
3. each product turns out to be effective with respect to a placebo rinse or water,
4. no contradicting conclusions with respect to clinical efficacies have been drawn on the basis of short term or long term studies except in the studies of Nygaard and Persson[12] and Affset and Rölla.[13]

In our study[1], which was designed as a short term investigation of 6 days rinsing in the absence of other oral hygiene, we found the highest efficacy for Hibident®, and a reasonable efficacy for Meridol®, while the other products showed significantly smaller effects. Therewith the importance of the present study becomes obvious again, i.e. given a suitable placebo control, every product can be proven effective, but a real comparison cannot be obtained.

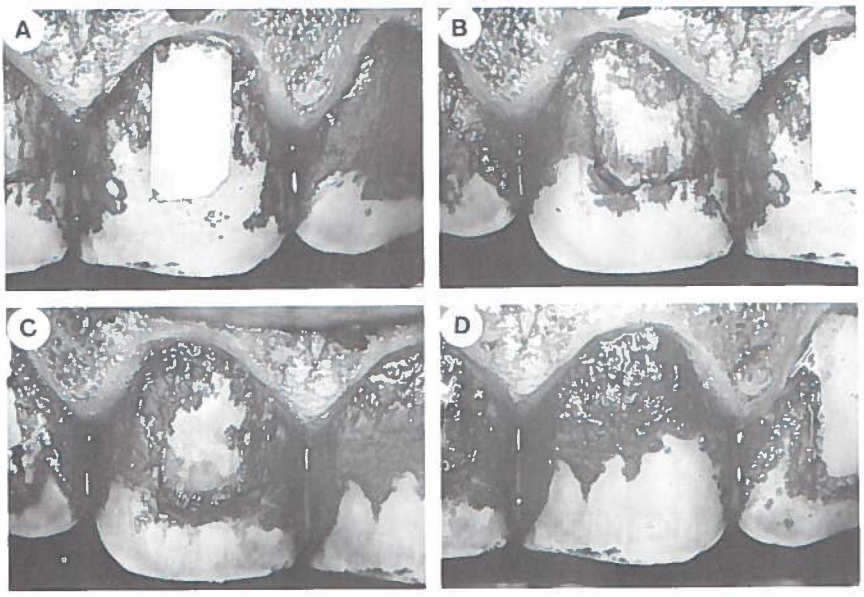

Fig. 7.1. Frontal photograph taken of polymer strips glued onto incisors of human volunteers. Photographs were taken after 9 days without oral hygiene and clearly show the influence of γ_s on plaque accumulation *in vivo*. (A Teflon®, γ_s = 20 $mJ.m^{-2}$; B Parafilm®, γ_s = 26 $mJ.m^{-2}$; C cellulose acetate, γ_s = 58 $mJ.m^{-2}$; D human enamel, γ_s = 58 $mJ.m^{-2}$ (from Quirynen *et al.*[15])).

Ad. 2:- possible working mechanisms of the mouthrinses based on physico-chemical effects.

Anti-bacterial properties of mouthrinses are still considered most important in facilitating clinical effects, and industrial research and development is concentrated on these issues. On the other hand the physico-chemical properties of mouthrinses, to our knowledge, have not yet been studies extensively. Meridol® for instance, is a product from which we had expected a major physico-chemical effect, i.e. a large tooth surface free energy reduction. Quirynen *et al.*[15] demonstrated clearly the effect

of surface free energies on the adhesion of dental plaque *in vivo*. In a clinical study a strong relation between the surface free energy and the amount of plaque adhering to polymer strips glued on the incisors of human volunteers was found. Fig 7.1 shows the amount of plaque accumulated on strips with different surface free energies.

After 3 days of no oral hygiene, FEP-Teflon® (γ_s = 20 mJ.m^{-2}) showed a very small part covered with plaque. As can be seen in Fig. 7.2, after 6 and 9 days of no oral hygiene, the low surface free energy substratum FEP-Teflon® still demonstrated a lower plaque accumulation than the higher energy substrata, cellulose acetate, (γ_s = 58 mJ.m^{-2}) and human enamel, (γ_s = 88 mJ.m^{-2}). A major plaque accumulation was found on human enamel.

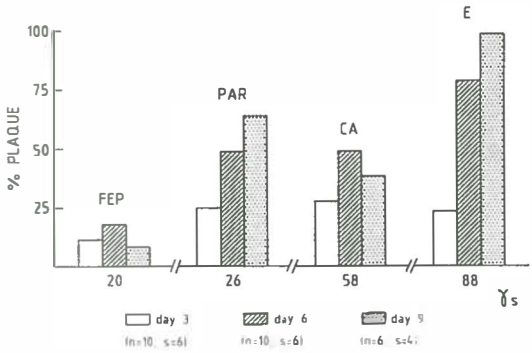

Fig. 7.2. The relationship between planimetric plaque (PP) score and the surface free energy after 3 days (first bar), 6 days (second bar) and after 9 days of no oral hygiene (third bar). FEP denotes fluorethylenepropylene (γ_s = 20 mJ.m^{-2}), PAR denotes Parafilm® (γ_s = 26 mJ.m^{-2}), CA denotes cellulose acetate (γ_s = 58 mJ.m^{-2}) and E denotes human enamel (γ_s = 88 mJ.m^{-2}) (from Quirynen *et al.*[15]).

Aminefluorides (AmF) are known to reduce the surface free energy of ground and polished human enamel; this effect seems however to be absent when aminefluorides are applied on pellicle coated enamel (Table 7.2).

Table 7.2. Surface free energies (γ_s, mJ.m^{-2}) and contact angles of water (H_2O,

degrees) and α-bromonaphthalene (α-Br, degrees) on ground and polished, as well as on pellicle coated human enamel after treatment with aminefluoride (AmF) for 10 minutes.[16,17]

Enamel	γ_s	Θ_{H_2O}	$\Theta_{\alpha\text{-Br}}$
Ground and polished	88 ± 8	48 ± 4	26 ± 1
Pellicle coated	104 ± 7	42 ± 5	31 ± 5
AmF treated	55 ± 4	83 ± 9	4 ± 7
Pellicle + AmF	110 ± 9	40 ± 8	23 ± 12
AmF + pellicle	92 ± 8	80 ± 8	35 ± 8

Obviously the adsorption of aminefluorides to the *in vivo* pellicle is different from the adsorption to polished human enamel *in vitro* without pellicle, since the surface free energy of pellicle coated enamel with AmF on top increased drastically. This phenomenon is probably caused by a different orientation of the polar group of the aminefluoride molecules towards the bare surface of enamel with and without pellicle. Furthermore, it is important to note that, although at first glance from Table 7.2 pellicle formation on low energy surfaces seems likely to destroy the effects of such a coating, Pratt-Terpstra *et al.*[18] showed that the surface free energy of the bare, uncoated substrata remain influential upon bacterial adhesion in spite of a marked influence of an adsorbed protein layer on both low and on high surface free energy substrata.

Considering the above information together with the results of the clinical study in Chapter 6, we have to conclude that the role of changes in γ_s caused by the ingredients of the rinses are of less importance for the rinse efficacy than previously

thought. This indicates that the amount of accumulated plaque at a given site is mainly determined by direct anti-bacterial effects of the rinses rather than by surface free energy changes induced by the rinse.

Ad. 3:- Future developments

Apart from the comparison of products this study has described several innovative pathways for evaluating working mechanisms of mouthrinses:

1. *in vitro* growth inhibition as an indication for clinically relevant concentrations of active components. This point may be particularly important as industries are working on the development of new anti-microbials, with a similar efficacy as chlorhexidine, but without the unfavourable side effects.
2. optimalisation of the penetration coefficient in order to increase penetration of a mouthrinse into the plaque and of their efficacy in periodontal pockets. Furthermore, penetration of a product may result in a loosening of the plaque. Looser plaque will be removed more easily by toothbrushing. In this respect we note, that Plax®, one of the first pre-brushing rinses on the market, is not throughout considered to be effective.[19,20]
3. monitoring the slow release of compounds from tooth structures by XPS and clinical contact angle measurements for i.e. quantification of the substantivity,
4. clinical registration of contact angles to determine the intra oral tooth surface free energy changes after using a mouthrinse. Future research in dentistry will probably be directed towards lowering the tooth surface free energy by applying a thin, non-visible permeable layer of low surface free energy material, to discourage bacterial adhesion and plaque formation. Such a coating may not only be important in dentistry, but also in shipping (non-toxic anti-fouling layers), food industries and in other processes in which bacterial adhesion creates

problems. This is, though challenging, one of the most difficult goals of mouthrinse development, as surfaces in the oral cavity are exposed to very dynamic conditions.

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CHAPTER 8

SUMMARY

The aim of the present study was to investigate physico-chemical properties of commercially available mouthrinses, as well as the effects of the rinses on the development of plaque and gingivitis.

The following rinses were investigated: Hibident® (containing 0.2% chlorhexidine), Meridol® (containing 125 ppm F^- as stannousfluoride and 125 ppm F^- as aminefluoride), Merocet® (containing 0.05% cetylpyridinium chloride), Veadent® (containing 0.03% sanguinarine), Listerine® (containing several phenolic compounds) and Prodent® (containing 0.05% F^- as sodiumfluoride).

In CHAPTER 2 a literature survey is given, emphasizing the compounds presently employed in mouthrinses. The use of chlorhexidine in rinses appears to be most effective and widespread. Furthermore, in this chapter the substantivity of a mouthrinse is described as an important factor. The substantivity is the prolonged association between compound and substrate during longer time intervals than would be expected upon simple mechanical retention.

In CHAPTER 3 the adsorption of several components present in commercially available mouthrinses to ground and polished enamel with and without salivary constituents, is investigated by X-ray Photoelectron Spectroscopy (XPS) and by contact angle measurements. XPS indicated a sizeable adsorption of both active and non-active components for all products investigated. After a rinse treatment all surface free energies (γ_s) increased, except for the stannousfluoride/aminefluoride containing mouthrinse. Probably the non-active components in the rinses cause this increase in γ_s, and are responsible for this thermodynamically unfavourable effect.

Despite the increase in γ_s all rinses are known to have a more or less plaque reducing effect, indicating that anti-bacterial rinse properties overrule surface free energy changes.

The physico-chemical properties of the rinses used in this study are presented in CHAPTER 4, being the surface tension, the *in vivo* measured tooth contact angle value after rinsing, the rinse viscosity, the rinse penetration coefficient, and the rinse acidity and buffercapacity. The penetration coefficient of a rinse determined by the surface tension, the contact angle and viscosity, is a measure for the ability of the liquid to penetrate into pores and capillaries, such as interproximal spaces, pockets and pits. The acidity, important for the influence of a rinse on de- and remineralisation, is obviously often a compromise between requirements set by taste, enamel re- and demineralisation and stability of the rinse. The properties of the various rinses differ greatly, particularly the penetration coefficient varied by a factor 1.8. Several products were found to be extremely acidic.

Bacterial growth inhibiting effects of the rinses are described in CHAPTER 5 using Hibident® containing 0.2 % chlorhexidine, as a positive control. Five bacterial strains, most likely responsible for the development of caries and gingivitis, namely *Streptococcus mutans* C67, *Streptococcus sanguis* CH3, *Veillonella alcalescens* V1, *Lactobacillus acidophilus* JP and *Actinomyces viscosus* C74, grown in batch cultures were evaluated. In analogy to the known concept of Minimal Inhibitory Concentration (for pure active components), we defined a Maximal Inhibiting Dilution for the whole rinse. With respect to Hibident®, the most effective product was Meridol®, followed by Merocet®, Veadent®, Listerine® and Prodent®.

In CHAPTER 6 the clinical effects of the rinses with respect to each other were evaluated on plaque indices, gingivitis indices and on the tooth surface free energy (γ_s). After a preparatory phase of 14 days all oral hygiene was stopped and a rinse was used twice a day for the following 6 days. Afterwards the Plaque Index, Gingival Index, the planimetric plaque index and γ_s were assessed. The composition of the plaque was determined at the beginning and at the end of the study. Hibident® and

Meridol® demonstrated the lowest PI and GI values after 6 days use. None of these products was able to alter the tooth surface free energy drastically, or to cause major shifts in the microbial composition of the plaque present.

In CHAPTER 7 it is illustrated that controversies exist in the literature concerning studies on mouthrinses. We conclude that it is necessary to develop mouthrinses with less side effects than chlorhexidine, with greater penetration potential and with major surface free energy reducing effects on tooth surfaces *in vivo*. Furthermore we emphasize the importance of the parameter γ_s in the adhesion of bacteria to tooth surfaces. Several clinical studies have convincingly shown that materials with a low γ_s accumulate less plaque than high γ_s substrata. It is discussed that the physico-chemical evaluation of mouthrinses as applied in this study, may offer new innovative pathways to develop improved rinse products.

SAMENVATTING

Het doel van dit onderzoek was het evalueren van physisch-chemische eigenschappen van commercieel verkrijgbare mondspoelmiddelen en hun effect op plaquevorming en gingivitis. De volgende middelen, die niet allen verkrijgbaar zijn in Nederland, werden gebruikt: Hibident® (bevat 0.2% chloorhexidine), Meridol® (bevat 125 ppm F^- als aminfluoride en 125 F^- als ppm tinfluoride), Merocet® (bevat 0.05% cetylpyridinium-chloride), Veadent® (bevat 0.03% sanguinarine), Listerine®, (bevat verschillende phenol-achtige stoffen) en Prodent® (bevat 0.05% F^- als natriumfluoride).

In HOOFDSTUK 2 wordt een literatuur overzicht gepresenteerd, waarin aandacht wordt besteed aan de verschillende stoffen en middelen die in mondspoelmiddelen verwerkt kunnen zijn. Het gebruik van chloorhexidine is het meest algemeen, ondanks verschillende nadelen die aan het gebruik ervan kleven. In dit hoofdstuk wordt benadrukt dat de zgn. 'substantivity' van een mondspoelmiddel erg belangrijk is. Hiermee wordt bedoeld de langere associatie tussen materiaal en substraat dan mag worden verwacht op grond van alleen retentie. Het is daardoor erg moeilijk om vanuit *in vitro* experimenten iets te voorspellen over de *in vivo* werking van een middel. Ondanks het vele onderzoek is er nog steeds geen anti-plaque middel gevonden dat zonder bezwaren voor langere tijd kan worden gebruikt.

In HOOFDSTUK 3 is de adsorptie van actieve en therapeutische stoffen aanwezig in mondspoelmiddelen met en zonder geadsorbeerde speekseleiwitten aan glazuur onderzocht met behulp van de XPS-techniek (X-ray Photoelectron Spectroscopy) en randhoekmetingen. XPS toonde een adsorptie aan van zowel actieve als niet-actieve stoffen uit de mondspoel-middelen. Na de verschillende behandelingen nam in alle gevallen de vrije opper-vlakte energie toe, behalve bij het aminfluoride/tinfluoride bevattende middel. Waarschijnlijk spelen de surfactants uit de mondspoelmiddelen voor dit uit thermo-dynamisch oogpunt ongunstige effect een belangrijke rol.

Ondanks de verhoging van de vrije oppervlakte energie hebben alle middelen toch een zeker plaque reducerend effect. De antibacteriële effecten van de middelen maskeren waarschijnlijk de effecten van de vrije oppervlakte energie veranderingen.

Physisch-chemische eigenschappen van mondspoelmiddelen worden belicht in HOOFDSTUK 4. Dit zijn de oppervlaktespanning, de *in vivo* randhoek, de viscositeit, penetratiecoëfficiënt, de zuurgraad en de buffercapaciteit. De penetratiecoëfficiënt wordt berekend uit de oppervlaktespanning van de vloeistof, randhoek en de viscositeit. Het is een maat voor het vermogen van een vloeistof om door te dringen in capillairen en kleine ruimtes zoals de interproximale ruimtes. De zuurgraad, belangrijk bij de de- en remineralisatie van het tandglazuur, wordt vaak bepaald door een compromis tussen smaak, de- en remineralisatie en stabiliteit van de vloeistof. Deze eigenschappen vertonen grote verschillen tussen de diverse mondspoelmiddelen, vooral de penetratiecoëfficiënt varieert met een factor 1.8. Sommige producten zijn extreem zuur.

Het groeiremmend vermogen van de mondspoelmiddelen wordt besproken in HOOFDSTUK 5. Hibident® werd gebruikt als een positieve controle. Er werden vijf bacteriestammen gebruikt, waarvan wordt aangenomen dat zij een belangrijke rol spelen bij het ontstaan van cariës en gingivitis. De Maximal Inhibiting Dilution werd bepaald aan de hand van batch culturen. Vergeleken met Hibident® was het effect van Meridol® het grootst, gevolgd door Merocet®, Veadent®, Listerine® en Prodent®.

In HOOFDSTUK 6 zijn de klinische effecten geëvalueerd van de mondspoelmiddelen op plaque, gingivitis en de *in vivo* vrije oppervlakte energie. Na een voorbereidende fase van 14 dagen werden alle mondhygiënische maatregelen gestopt, en werd er alleen gespoeld met één van de middelen gedurende 6 dagen, 2x per dag. Daarna werden de Plaque Index, Gingiva Index, de Planimetrische Plaque index en de vrije oppervlakte energie bepaald. De samenstelling van de plaque werd aan het

begin en het einde van de experimentele periode geëvalueerd. Geen van de produkten was in staat de vrije oppervlakte energie sterk te beïnvloeden, of grote veranderingen te veroorzaken in de samenstelling van de plaque.

In HOOFDSTUK 7 wordt geïllustreerd dat er controverses bestaan in de literatuur betreffende mondspoelmiddelen. Er wordt geconcludeerd dat er behoefte is aan mondspoelmiddelen die qua werking gelijk zijn aan chloorhexidine, maar minder nadelige effecten vertonen. De nadruk wordt gelegd op het belang van de rol die de vrije oppervlakte energie speelt bij de hechting van bacteriën aan tandoppervlakken. Er worden verschillende innovatieve oplossingen beschreven om nieuwe, verbeterde mondspoelmiddelen te ontwikkelen.

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